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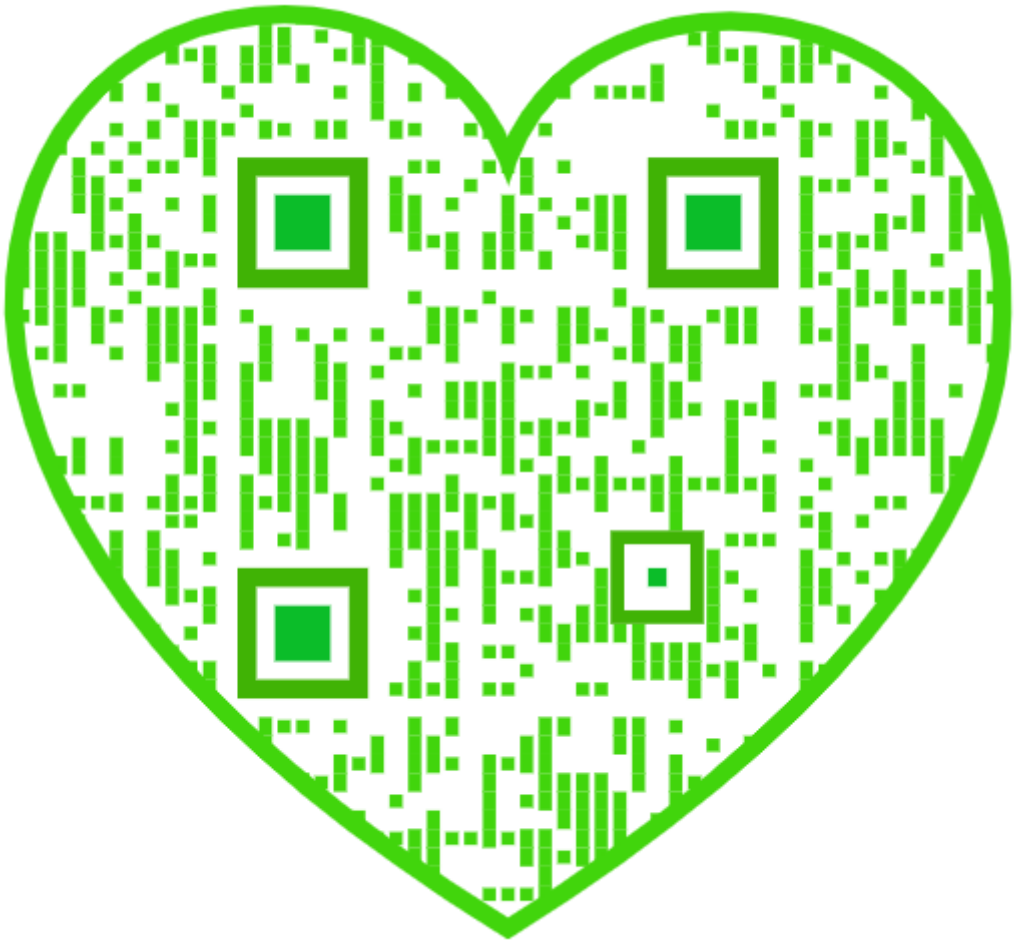
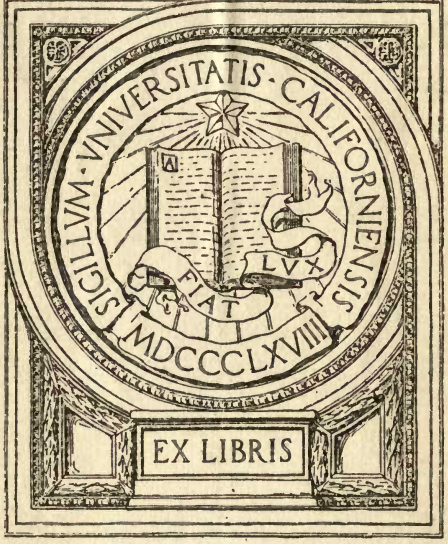


IN MEMORIAM
Richard M. Holman



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A COURSE OF PRACTICAL INSTRUCTION

IN

BOTANY.

A

COURSE OF PRACTICAL INSTRUCTION

IN

BOTANY

BY

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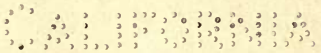

WITH A PREFACE BY

W. T. THISELTON DYER, M.A., C.M.G., F.R.S., F.L.S.,

ASSISTANT-DIRECTOR OF THE ROYAL GARDENS, KEW.

PART I.

PHANEROGAMÆ—PTERIDOPHYTA.

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IN MEMORIAM
Richard M. Holman

PREFACE.

A FEW words may be said to explain the origin of the work of which the present portion is a first instalment. In 1873 I was invited by the Science and Art Department to conduct a course of instruction in what is now the Normal School of Science at South Kensington. It was a condition of the undertaking that the instruction should be carried on continuously from day to day and throughout the working hours of each day. My friend Mr. Lawson, late Professor of Botany at Oxford, was so good as to give me his assistance. We had the use of Professor Huxley's convenient and well-appointed laboratory, and we determined to attempt a course of instruction which should embrace the leading morphological facts of every important type in the vegetable kingdom. We, in fact, resolved to adopt exactly the same plan of work as Professor Huxley in his own teaching had found convenient for the animal side of morphology.

At this time, as far as I am aware, no previous attempt had been made in this country to give an extended

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course of botanical instruction of this kind. Professor Lawson and myself found our own difficulties scarcely less considerable than those of the students. The interest, however, which the novelty of the new method of work excited in the class soon became very obvious. The enthusiasm of the more skilful students at once stimulated and assisted us, and at the conclusion of the course we found that there was scarcely anything of importance in the rather comprehensive range which had been attempted which the students had not been able to study, examine, and draw for themselves.

This course was an experiment. It was repeated at irregular intervals during the next few years. It gradually took a more systematic shape, and with the appointment of Mr. Bower as Lecturer on Botany in the Normal School, it is likely, I think, to settle down into a permanent system of instruction.

I had always hoped to put together the results of the experience in teaching methods acquired at South Kensington in the form of a handbook, which should save teachers who wished to follow our example from much of the trouble and difficulty which I, and those who, at different times, have taught in this way, have had to face. But, in the meanwhile, I had been drawn off to administrative duties which have left a steadily diminishing leisure for purely scientific work. Fortunately, my friend Mr. Bower was willing—and with far greater competence—to take up the task which I was unable to perform, and to him are entirely due the

laboratory instructions for studying the different types selected. Dr. Vines has very kindly supplied the chapters on methods and on the morphology of the cells. But besides this he has at every step given the assistance of his own extensive experience in practical teaching.

It had been our intention to preface the directions for the study of each type with a short account, in language fairly intelligible to the general reader, of its salient morphological facts. This would have represented the brief lecture with which the work of each day began in the course as originally organised. To carry out this intention would have postponed the publication of the teaching directions already prepared by Mr. Bower, and, in justice to him, it has seemed the best course to issue what is already finished without delay. It is intended to follow the present part with another, which will comprise the remaining types of the vegetable kingdom. Should the book be found as useful to students as it is hoped may be the case, I look forward to seeing the original scheme upon which it was planned still carried out in a future edition.

W. T. THISELTON DYER.

ROYAL GARDENS, KEW,
December 1884.

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PRACTICAL BOTANY.

I.

Methods and Reagents.

A.—*Making Preparations.*

Preservation of Material.—In many cases it is possible, and even preferable, to use fresh material, but it is often convenient to keep it for a time; the best liquid for this purpose is ordinary methylated alcohol, in such quantity as to completely cover the material. It must be remembered that this will extract the green colouring matter (chlorophyll) from the material immersed in it, as well as resin and other substances.

Hardening.—It is not necessary, for the general study of the histology of plants, to harden them, for the tissues are usually sufficiently firm to admit of their being cut satisfactorily. In the case of exclusively parenchymatous tissues, especially those of cellular plants, it is necessary to harden them somewhat, and for this purpose dilute alcohol (50 per cent.) may be used.

When it is desired to study the structure of the protoplasm and of the nucleus, special methods must be employed for hardening them, or rather, for fixing them as nearly as possible in the condition in which they were during life. For this purpose the fluids mentioned below must be used. Care must be taken that the objects are of small size, that the quantity of hardening fluid is very large relatively to the bulk of the object, and that the fluid has ready access to all parts of it.

The following are the best fluids for this purpose :—

1. Absolute alcohol.
2. Picric acid (saturated solution in water).
3. Chromic acid (0·1—0·5 per cent. solution in water).
4. Osmic acid (·1 – 1 per cent. solution in water).

These reagents can only be applied to fresh material.

The following is a useful method for preparing sea-weeds : to a quantity of saturated solution of picric acid in sea-water add three or four times its volume of sea-water, and treat the tissue with it for ½ hr.—2 hrs. : then treat successively with 30, 50, 70, and 90 per cent. alcohol.

When absolute alcohol is used, the object may be kept in it for an indefinite period. Such treatment generally makes the object brittle ; this may be remedied when the object is to be mounted in glycerine by placing it, for at least twenty-four hours before it is to be cut, in a mixture of glycerine and absolute alcohol in equal parts, leaving it exposed to the air so that the alcohol may gradually evaporate. The glycerine slowly saturates the object and restores its consistency. This

can of course only be done when the sections are to be mounted in glycerine.

When picric or chromic acid is used, the object should be immersed in it for several hours; the length of time varies with different material, and, in the case of chromic acid, with the strength of the solution used, from a few minutes to twenty-four hours. The objects must then be washed with dilute alcohol (50 per cent.), and then placed in stronger alcohol (70 per cent.), and finally in absolute alcohol (or 90 per cent.), which must be changed so long as any colour is still extracted from the objects. They may be preserved in this for future use.

When osmic acid is used, the fixing effect is produced much more rapidly; in the case of simple structures, such as unicellular or filamentous Algæ a few minutes (5—15) generally suffices; in the case of more complex structures, such as ovules, sporangia, growing-points, &c., the object may be left in the acid till it looks black on the exterior: it must be then well washed with dilute alcohol (50 per cent.), and left in it for some time, and be then removed to 70 per cent. The sections are best mounted in dilute glycerine. In some cases osmic acid produces an excessive blackening of the cells, which can be removed by treatment with chlorine-water.

It is advisable in cases in which the cell-walls tend to swell up excessively (as in many Algæ) to use solutions of picric, chromic, and osmic acids, to which an equal volume of absolute alcohol has been added.

Of these hardening reagents the most serviceable are absolute alcohol, or 90 per cent. alcohol, and picric acid.

Cutting Sections.—A sharp razor is the best cutting instrument. Care must be taken to keep the object and the razor wet during the process of cutting, in order to avoid the entrance of air into the tissue, and to prevent adhesion of the section to the razor. When fresh material is cut, water or very dilute alcohol may be used for this purpose, but if material which has been hardened is cut, it is advisable to use alcohol of the same strength as that in which the material has been preserved.

When a successive series of sections of an object is required, a microtome may be used.

Imbedding.—The objects are frequently so large that they may be held in the hand whilst they are being cut. If they are too small for this it is convenient to imbed them in some substance.

The simplest method is to fix the object into a slit in a piece of pith. Elder-pith is the best.

When the sections are to be made with a microtome, it is more convenient to imbed in some easily fusible substance; by this means also the position of the object is less likely to be distorted in the process of cutting. Many mixtures of waxy and fatty substances are used for this purpose, of which the following is perhaps the best:—

Solid paraffin (melting-point about 58° C.): 2 parts.
Vaselin: 1 part.

These must be melted together and well stirred. The resulting substance is sufficiently transparent to enable the exact position of the object to be ascertained; it is easy to cut, and it is readily soluble in carbolic acid and turpentine. The relative proportions of paraffin and

vaselin may be varied somewhat to suit the object; a softer mixture is produced by increasing the proportion of vaselin. For soft objects cacao-butter, which has the advantage of being soluble in ether or chloroform, is useful.

The method of imbedding is to make a cavity in a piece of the substance sufficiently large to contain the object, which must have been previously washed with alcohol to remove all traces of water from its surface; a small quantity of the substance is then melted and poured into the cavity so as to surround and cover the object. When it is cold it may be cut.

Another method of imbedding is to moisten the object in water, and then suspend it by means of a pin attached to a thread in some white of egg, which has been previously well shaken up, and then strained through muslin. The white of egg should be in an evaporating dish. The object should be left thus suspended for some hours, so that the white of egg may come into close contact with all parts of it. Heat is then applied by means of a water-bath, and the white of egg coagulates. The part surrounding the object is now cut out and hardened in alcohol for some days. This method is useful for making sections of buds and flowers.

It is important to keep the imbedded objects wet with alcohol during the process of cutting, in order to prevent the drying-up of the object, and its consequent contraction away from the substance in which it is imbedded.

A third method of imbedding is very useful when it is desired to obtain sections of very small objects, such

as spores, pollen-grains, &c. This is effected by means of gum. A thick layer of strong clean gum is laid on the flat surface of a piece of pith; this is allowed to become nearly dry; and then the pollen grains or spores are dusted on to it; these are then covered with another thick layer of gum, and the whole is allowed to dry. Sections are now made of the dried gum, and, on their being placed in water, the gum is dissolved, and the sections of the pollen-grains or spores are set free.

Staining.—It is often useful to stain sections in order to bring out certain points in their structure, which are difficult to observe under ordinary circumstances. A great number of colouring matters have been used for this purpose, among which may be mentioned as the most useful:—Hæmatoxylin, Carmine, Cochineal, Gold Chloride, various preparations of Aniline, such as Safranin, Nigrosin, Fuchsin, Methyl-green, Eosin, and Methyl-violet.

Staining is best performed by placing a few drops of the staining-fluid in a watch-glass and immersing the sections in it. The exact strength of the fluid, and the time of exposure of the sections to its action varies in each case, and must be ascertained by preliminary trials. As a rule, when differentiated staining is desired, the best results are obtained by using a dilute solution, and by exposing the sections for a long time to its action.

Hæmatoxylin.—A number of preparations of this colouring-matter are in use; of these the following are those generally employed for vegetable tissues.

1. Alum solution of Hæmatoxylin.—Dissolve 0·35 grammes of hæmatoxylin in 10 c.c. of water, and add to it a few drops of a

solution of alum consisting of 1 gramme of alum to 1 c.c. of water.

2. Kleinenberg's hæmatoxylin.—Saturate some 70 per cent. alcohol with calcium chloride ; let the mixture stand for twelve to twenty-four hours over alum, shaking occasionally ; add eight parts of 70 per cent. alcohol ; filter, and then add a solution of hæmatoxylin in absolute alcohol until the liquid has a purple-blue colour ; let it stand in a corked bottle exposed to sunlight for about a month ; it is then fit for use. The liquid is to be diluted as required with alum solution.

3. Expose a few crystals of hæmatoxylin to the action of gaseous ammonia in a watch-glass under a bell-jar : then add water, and a good colouring fluid is obtained. The disadvantage of this is that it has to be freshly prepared every time it is required.

The alum-solutions will stain all parts of the cell, including the cell-wall. Their especial uses are (*a*) to make the cell-walls more evident when they are naturally transparent and colourless ; (*b*) to stain the protoplasm, so as to make its intimate structure apparent ; (*c*) to stain the nucleus, so as to demonstrate its presence and to show up its structure.

The ammoniacal solution is especially adapted for differentiated staining. If a dilute solution be used, the first thing to become stained is the chromatin of the nucleus, then, after a time, the rest of the nucleus (achromatin), then the protoplasm. The cell-walls do not stain with this fluid, or only slightly. Kleinenberg's hæmatoxylin stains in a few minutes, whereas the alum-solution is much slower in its action.

Hæmatoxylin may be used either for fresh material, or for sections which have been previously hardened with alcohol, or with picric or chromic acid. In the latter case the sections must be washed repeatedly in distilled water to remove every trace of the acid, which, if present, would interfere with the proper action of the hæmatoxylin. If the section becomes too deeply stained, as sometimes happens when the alum-hæmatoxylin is used, the excess of colouring-matter may be removed by washing with watery solution of alum.

Sections stained with alum or with Kleinenberg's hæmatoxylin are to be mounted in Canada balsam (or Dammar). Those stained with the ammoniacal solution are to be mounted in glycerine.

Carmine.—The two best preparations of carmine are those of Beale and Thiersch: carmine possesses, however, but little differentiating power.

1. Beale's Carmine—To prepare this 0·6 gramme of carmine is dissolved in 2 c.c. of boiling solution of ammonia; the solution must then stand for an hour or so to cool, and to allow of the escape of the superfluous ammonia; to the solution are added 60 c.c. of distilled water, 60 grammes of glycerine, and 15 grammes of absolute alcohol. The mixture must be allowed to stand for some time; it is then to be filtered.

2. Thiersch's Carmine—Four grammes of borax are dissolved in 56 c.c. of distilled water; to this 1 gramme of carmine is added, and then twice its volume of absolute alcohol is added to the liquid. After filtration the liquid is ready for use.

Carmine readily stains the protoplasm and the nucleus; Thiersch's preparation is especially useful for bringing out the structure of the nucleus. It can very well be used for sections which have been previously treated with picric, chromic, and osmic acids. The time during which the section is to be exposed to its action varies very much, as is the case with hæmatoxylin. The rule is in both cases, that the most satisfactory results are obtained by a prolonged immersion in a dilute solution. In case of overstaining, the section may be washed for a moment in water, to which a trace of ammonia has been added.

Preparations stained with carmine are best mounted in glycerine.

3. Picro-carmine (or ammonium picro-carminate) is another useful preparation of carmine. It is prepared by adding a strong ammoniacal solution of carmine to a quantity of concentrated solution of picric acid in water, until a precipitate begins to be formed; it is then evaporated to about one-fifth of its bulk filtered, and the filtrate is evaporated to dryness. The crystalline residue is dissolved in water so as to make a 5 per cent solution, and this may be diluted as occasion requires.

Another method (Gage) is to dissolve a quantity of picric acid in 100 parts of water, and an equal quantity of carmine in 50 parts of solution of ammonia; these are then mixed, filtered, evaporated to dryness, and the residue dissolved in 100 parts of water.

Picro-carmine is used especially for staining nuclei, the staining being more uniform than when carmine alone is used: it has this further advantage, that a prolonged exposure to it does not produce overstaining, as is the case with the other preparations of carmine. The objects should be previously kept for some time in absolute alcohol. If it is desired to retain the double staining which this reagent produces, the sections must be mounted at once in glycerine; but if the carmine staining only is required, the sections must be washed in water, which will dissolve out the picric acid. When stained sections are mounted in glycerine, a small quantity of picro-carmine must be added to the glycerine in order to preserve the colours.

The various preparations of carmine can be used as well for tissues which have been hardened in chromic, picric, or osmic acid, as for fresh tissues, but the former stain less readily.

4. Cochineal.—The ordinary preparations of carmine frequently fail to give good results, especially when the tissue has been previously treated with chromic acid. Other preparations of the same colouring matter made directly from the cochineal insect have therefore been employed.

1. Alcoholic Solution.—A quantity of finely powdered cochineal (best grey) is extracted for several days with 70 per cent. alcohol; the liquid is filtered off and is ready for use.

2. Solution in water.—Seven grammes of cochineal and an equal quantity of burnt alum are rubbed up together in a mortar until the whole is a fine powder: the powder is then added to 700 c.c. of

distilled water; the whole is then boiled, and evaporated to 400 c.c.: when it is cool a trace of carbolic acid is to be added, and then the liquid is passed two or three times through a filter. A dirty-red substance remains on the filter, and the filtrate is a clear fluid, thin layers of which appear red and thicker layers violet. This fluid will keep well for some months, but every now and again a trace of carbolic acid must be added to it, and it must be filtered.

Both these preparations give good results, the differentiation being very marked. In using the alcoholic solution, the sections must be first soaked in 70 per cent. alcohol before they are placed in the staining liquid: it is also necessary, when sections are to be stained, to dilute the solution considerably with 70 per cent. alcohol. The watery solution acts very rapidly, staining fresh or alcohol material in a few minutes (3—5).

The solution of cochineal in water stains especially the bast-fibres of vascular bundles. In some cases the whole of the wood stains, but if the section be treated with dilute hydrochloric or sulphuric acid, the colour will be removed from all the cell-walls except those of the bast-fibres.

Gold-chloride, in 0·5 per cent. solution in water, has been employed for staining Fungi. They must remain in it from one to six hours, and be mounted in dilute glycerine.

Aniline colouring-matters.—A large number of these have been employed, only the more important are mentioned here; they all stain rapidly.

1. **Safranin.**—This is used in solution in absolute alcohol. It is especially adapted for staining sections which have been previously hardened with chromic or picric acid; it is not quite so good for those which have been treated with osmic acid. The sections must be well washed in distilled water, and then placed in a small quantity (1 c.c.) of the saturated alcoholic solution mixed with an equal volume of distilled water; they require to be left for several hours in the staining fluid. They must then

be removed, and washed for a short time in alcohol ; then they must be placed in absolute alcohol and kept there until they appear transparent. The sections can now be mounted in distilled water in order to see if the results are satisfactory, or, if they are to be preserved, they must be cleared by means of oil of cloves, and mounted in Canada balsam or Dammar.

By this means very successful preparations of the structure of nuclei can be obtained.

2. **Fuchsin.**—This is used in alcoholic solution. It is useful for bringing out the structure of thickened cell-walls. The sections must be previously treated with alcohol. It is also a good reagent for corky tissue. When a section is stained and is then washed with absolute alcohol, the coloration is removed from all parts excepting the corky tissue.

3. **Methyl-green.**—A tolerably strong alcoholic solution of this is used. The sections of the object, which must have been previously kept in absolute alcohol, are to be treated with the staining-fluid for from 5—25 minutes, then quickly washed with distilled water, and mounted in glycerine. The nucleus stains of a green or bluish-green colour, the protoplasm remaining uncoloured. It is especially good for staining nuclei which are dividing. It has been used for staining chlorophyll-corpuscles, and is also useful in bringing out the nuclei and protoplasm in the cells of Fungi, which have been previously preserved in absolute alcohol and in glycerine.

Strasburger recommends the following method for obtaining preparations of nuclei :—A section of the fresh tissue is mounted in 1 per cent. acetic acid solution, to which a little methyl-green has been added ; the nuclei are fixed almost instantaneously.

4. **Methyl-violet.**—This is used in concentrated alcoholic solution. It is especially useful for staining bacteria. A few drops of the solution are added to 15—20 c.c. of distilled water, and a drop or two of the mixture should then be placed on the bacteria-membrane (zoogloea), and be allowed to remain there for a short time until the membrane appears to be coloured : if the solution used is too strong, the substance between the bacteria will become stained. The colouring-matter is then washed off with distilled water, or better with a 10 per cent. solution of acetate of potash. The preparation may then either be allowed to dry in the air and

be then mounted in Canada balsam, or it may be mounted in a 50 per cent. solution of potassium acetate in water.

A useful preparation of methyl-violet is the following :—Some of that substance is dissolved in strong sulphuric acid, forming a brownish-green solution : on the gradual addition of water the violet colour reappears. This is especially useful for sieve-tubes. If a section be treated with this fluid for a short time, and be then washed with water, it will be found that the cell-walls have become swollen and transparent, that the protoplasm has become deeply stained, and that the sieve-plates are very well brought out. Lignified tissues treated with this fluid assume a yellow colour, as they do when treated with aniline sulphate.

5. Hanstein's **Aniline-violet.**—This is prepared by dissolving equal parts of fuchsin and methyl-violet in alcohol. It stains cellulose cell-walls of a faint violet colour, and lignified cell-walls red. It is especially useful for bringing out the different parts of the bast, since the bast-fibres stain red, whereas the sieve-tubes and the parenchyma scarcely stain at all. The protoplasm is stained pink ; amyloid substances, gums, and nuclei stain different shades of red, resins blue, and tannin brick-red.

6. Hoffmann's **Blue.**—Used in solution in dilute alcohol slightly acidified with acetic acid : it is a useful reagent, inasmuch as it stains the protoplasmic cell-contents and not the cell-wall : it stains also the callus which closes the perforations of the sieve-plates during the winter in perennial plants. (Water blue is almost as good a reagent.)

7. **Methylene blue.**—Used in solution in water : stains the cell-wall but not the protoplasm.

To produce the differentiated staining mentioned in 6 and 7, the preparations must be washed in water after staining, and also before staining if the material has been previously kept in alcohol.

8. **Alizarine.**—Many of these aniline-dyes will not stain the protoplasm of Fungi. Alizarine will do so at least in some cases.

9. **Eosin.**—Used in strong alcoholic solution for demonstrating the structure of sieve-tubes, as it stains the protoplasm deeply : a solution in water may also be used.

10. **Corallin** (rosolic acid).—A solution in 30 per cent. sodium carbonate colours lignified tissue, the callus of sieve-tubes, and starch grains pink.

To the detailed instructions given above, the following general remarks may be added. All the above-mentioned staining-fluids may be used for protoplasm and nuclei.

The stain produced by aniline-colours is apt to fade, so that they are not to be recommended for preparations which are to be kept for a long time. The staining of hæmatoxylin also fades, but more slowly. In order to prevent fading, the preparations should be kept in the dark.

Clearing the preparations.—If it is not desired to observe the details of structure of the protoplasm or of the nucleus, the best clearing agent is a solution of potash, either in water or alcohol. The most generally useful is the 5 per cent. solution made by dissolving five grammes of solid caustic potash in 100 c.c. of distilled water. The alcoholic solution is made by adding strong alcohol (ordinary methylated alcohol will do) to a quantity of a concentrated solution in water until a precipitate begins to be formed. The mixture must then be well shaken, and allowed to stand and settle for twenty-four hours; the clear fluid is then poured off. For use a mixture of equal parts of this solution and of distilled water may be made.

The clearing action of potash is due to the swelling of the cells and their contents, so that they become more transparent; at the same time it dissolves many of the granules in the protoplasm, and saponifies the oil-drops. The swelling caused by the action of the solution in water is often too great, especially when it is desired to see the cell-walls distinctly; this difficulty may be got over by the use of the alcoholic solution.

After treatment with the aqueous solution, the sections should be washed in distilled water, and after treatment with the alcoholic solution in dilute alcohol; the sections, in both cases, should be mounted in glycerine.

Another method of clearing, which is especially recommended for obtaining good preparations of growing points, is to treat sections with calcium chloride. The sections are placed on a slide in a drop of water, and are then covered with some dry powdered calcium chloride; the slide is then warmed over the flame of a spirit-lamp until the water has nearly all evaporated; a drop or two of water is now placed on the sections, and they are to be mounted in glycerine.

In the case of tissues, which have been hardened in alcohol, with or without treatment with other hardening agents, another method of clearing may be used. The sections, after staining, if that is desired, should be placed for a few minutes in absolute alcohol; they should then be transferred to a watch-glass, containing either a mixture of turpentine and creosote (four parts of the former to one of the latter), or some oil of cloves; sections which have been stained with aniline dyes are best cleared by cedar-wood oil; they should be left in this for a short time, until they appear to be quite transparent, and should then be mounted in a drop of Canada balsam or Dammar.

Mounting.—For the observation of the coarser features of the histology of plants, it suffices to mount the sections in a drop of water, or, in certain cases, in a drop of alcohol. This is the only possible method when micro-chemical observations have to be made. Sections of objects which have been hardened, or otherwise

specially prepared, and which it is desirable to preserve, should be mounted in glycerine, or in glycerine-jelly, or in Canada balsam or Dammar. Glycerine and glycerine-jelly may be used for sections which have been prepared by any of the methods described above. Dilute glycerine should be used for this purpose, consisting of a mixture of pure glycerine with an equal bulk of water. The cases in which these media are especially suitable have been mentioned. Only those sections which have been treated with absolute alcohol, and either oil of cloves or the mixture of turpentine and creosote can be mounted in Canada balsam or Dammar.

When preparations are mounted in glycerine-jelly, a trace of carbolic acid should be added in order to prevent the growth of Fungi. The sections should be previously soaked in glycerine so as to remove water or alcohol from them.

In order to make the preparations mounted in glycerine quite permanent, the cover-slip should be fixed to the slide by applying a coating of gold-size or Brunswick black round its edge with a brush. Care should be taken that no glycerine is on the slide outside the cover-slip; if any is there it should be removed by means of blotting-paper before applying the varnish.

It is better to varnish Dammar preparations in this way also; but it is not necessary for those in Canada balsam.

Preparations of green parts of plants in glycerine lose their colour. These may be best put up in a drop of a strong solution of potassium acetate, or of aluminium acetate. The cover-slip must be fastened down as above described.

It is often desirable to observe objects in the living state for a considerable time under the microscope. This must be done in a **moist chamber.** A moist chamber may be readily constructed as follows: A piece of thick rough cardboard is cut to the size of the glass slide, and a circular hole is punched out of the middle of it of such a size as to be covered by a cover-slip. The piece of cardboard is then soaked in water (or boiled in water when pure cultures of Fungi are to be made), so as to saturate it, and placed on the glass slide. A drop of water (or solution as described below), is placed on the cover-slip, the object is immersed in it, and the cover-slip is then inverted over the hole in the piece of cardboard. Thus the object is suspended in a drop of liquid on the under surface of the cover-slip. Any loss from the chamber by evaporation is prevented by occasionally wetting the cardboard on the slide.

The liquid to be used will of course vary with the nature of the object to be observed. In the case of Algæ, water may be used; in the case of Fungi, decoctions of various organic substances (fruits, animal tissues, &c.), or a solution of sugar, according to the habit of the Fungus. For observing the germination of the spores of Mosses and Ferns, water will suffice; but in the case of pollen-grains a solution of sugar is necessary (1—20 or even 30 per cent., the concentration being different for different plants); for observing the process of cell-division in the hairs on the stamens of Tradescantia, a 1 per cent. sugar solution may be used.

B.—*Micro-chemical Reagents.*

Besides the fluids which are used for hardening and staining the tissues, a considerable number are employed, which, on account of the characteristic effects produced by their action on cell-walls and cell-contents, may be regarded as chemical tests for the various substances which may be present. The following are the principal reagents which are used in this way: the mode of preparing them is also given, and some indication of their uses; but this latter subject is more fully treated in the next chapter.

I. **Acids.**

Sulphuric acid.—This is used either concentrated or dilute (1 to 3 of water). It causes, in either case, the swelling-up of cellulose cell-walls, starch-grains, &c.; when cellulose cell-walls which have been previously saturated with solution of iodine are treated with sulphuric acid, they turn blue.

Concentrated sulphuric acid dissolves cellulose and starch, but cuticularised cell-walls and the middle lamella of lignified cells resist its action. It is used with cane-sugar, as a test for proteids, and with aniline sulphate as a test for lignin.

Nitric acid.—It colours cuticularised cell-walls and proteids yellow; it also causes swelling-up of cellulose and of lignified cell-walls. It is useful for dissolving the crystals of calcium oxalate which are frequently present in the cells. It is used with ammonia as a test for proteids (xanthoproteic reaction), and with potassium chlorate as a test for suberin, and as a macerating fluid.

Hydrochloric acid.—Used, with aniline chloride phloroglucin, or carbolic acid, as a test for lignin. By itself it turns lignified cell-walls yellow; when its action is prolonged, the cell-walls become violet, owing to the presence of various substances such as phloroglucin, coniferin, and pyrocatechin.

Chromic acid.—A strong aqueous solution of this acid dissolves lignified and cellulose cell-walls; cuticularised cell-walls resist its action; but they become very transparent, and may be easily overlooked. A dilute solution brings out the stratification of cell-walls very clearly.

Acetic acid.—This is used as a dilute aqueous solution (1 per cent.). It dissolves crystals of calcium carbonate; it causes swelling-up of cell-walls, starch-grains, &c.; it brings out nuclei very clearly; it is useful as a corrective after treatment of a preparation with potash.

II. **Alkalies.**

Potash.—This may be used either in a dilute or a concentrated solution in water. The dilute solution is chiefly used for clearing preparations, as already described. It causes cell-walls, starch-grains, &c., to swell up very much, and it dissolves proteid crystalloids, and most aleurone-grains. It gives a reddish colour to cells in which tannin is present. It may be used as a macerating fluid; when woody tissues are boiled in potash, the cells of the vascular bundles become more or less isolated, for the lignin of their walls undergoes solution. It dissolves inulin.

The concentrated solution is used as a test for suberin. When sections of cork are boiled in strong

potash, the suberin escapes in the form of yellow viscid drops; when the sections are only slightly warmed in potash solution the cuticularised cell-walls assume a yellow colour.

Potash is also used, together with copper sulphate as a test for proteids, and for various kinds of sugar.

Ammonia..—The solution in water is often used instead of potash for clearing preparations, as its action is less intense. It is used, together with nitric acid, as a test for proteids, and with copper sulphate as a solvent for some forms of cellulose.

III. **Non-Metallic Elements.**

Iodine.—This is one of the most useful micro-chemical reagents. It is used in solution, in water, or alcohol, and in the chloride of zinc mixture.

1. Solution in water. Dissolve a small quantity of potassium iodide in the requisite quantity of water; then dissolve iodine in it until the liquid has a dark sherry colour. This may also be prepared by diluting the *liquor iodi* of the pharmacopœia.

2. Alcoholic solution. Dissolve iodine in alcohol until it has a dark sherry colour. This may also be prepared by diluting with alcohol the *tinctura iodi* of the pharmacopœia.

Iodine stains proteid substances brown, cellulose faintly yellow (as a rule, see next chapter), cuticularised and lignified cell-walls yellow, gum purple, starch blue (only in the presence of water).

Iodine is used as a micro-chemical test for starch and for cellulose. The blue colour which it gives with starch, and the conversion of the faint yellow colour of a cellulose cell-wall stained with iodine into blue when

it is treated with sulphuric acid, are characteristic. The cellulose reaction is also given with the chloride of zinc mixture.

IV. **Inorganic salts.**

Sodium chloride (common salt).—This is used both in dilute (10 per cent.), and in saturated solution in water as a solvent for the proteid crystalloids.

The 10 per cent. solution is used for producing plasmolysis.

Ferrous sulphate.—Used in dilute solution in water, to which a drop of nitric acid has been added, as a test for tannin.

Potassium bichromate.—Used in dilute solution in water as a test for tannin; used also (in 1 per cent. aqueous solution) for hardening tissues.

Potassium chlorate.—Used, together with nitric acid, as a macerating agent.

Copper sulphate.—Used in very dilute solution in water; the blue colour of the solution must be only just perceptible. It is used, with potash, as a test for some kinds of sugar, and for proteids. It is used also in the preparation of ammoniacal solution of cupric hydrate, which dissolves pure cellulose. For the preparation of **Fehling's fluid** the following directions are given in Foster's *Practical Physiology*:—

(*a*). Dissolve 34·65 grm. of pure crystallised cupric sulphate in about 160 c.c. of distilled water.

(*b*). Dissolve also 173 grm. of pure crystallised potassic-sodic tartrate in 600 to 700 grm. of sodic hydrate sp. gr. 1.12.

Add (*a*) to (*b*) stirring well to cause a thorough mixture, and dilute with distilled water to a litre.

Fehling's fluid should be fresh made whenever it is required, since it decomposes on keeping; it will keep some little time if

kept in a cool place in the dark, and in completely filled, well-closed bottles (Hoppe-Seyler).

The solution (*b*) may be prepared, and kept for adding to (*a*) freshly prepared when required.

Before using a kept solution to test for sugar, always boil a little of it by itself to see if any reduction will take place.

From 1 c.c. of this solution the copper is completely reduced by ·005 grm. of grape-sugar.

V. Organic substances.

Alcohol.—Used as a solvent for various substances, such as fats, oils, resins, colouring-matters, &c., and as a precipitant for various substances. It has a peculiar action upon some proteid crystalloids.

Ether.—Used as a solvent for wax, fats, resins, &c.

Cane sugar.—The concentrated aqueous solution is used, together with strong sulphuric acid, as a test for proteids. A dilute (1 per cent.) solution is useful for mounting living cells for observation under the microscope.

Alkanet.—The alcoholic extract, or better, an alcoholic solution of alkannin, is used as a test for resin and caoutchouc: a fresh solution must be prepared on each occasion.

Phloroglucin.—Used in alcoholic or aqueous solution as a test for lignin. The section is first treated with hydrochloric acid and then with solution of phloroglucin: the lignified cell-walls assume a bright red colour.

If phloroglucin cannot be obtained, it may be replaced by an extract of cherry wood. Shavings of young cherry-branches are extracted with alcohol for twenty-four hours to remove chlorophyll and other substances; then the alcohol is poured off. The shavings, after being pressed, are extracted for several days with alcohol,

the alcohol extract is poured off and filtered and then evaporated nearly to dryness, until a piece of coarse blotting paper moistened with it and treated with hydrochloric acid turns violet. The extract is then ready for use. It gives a violet colour to lignified cell-walls, as it contains other substances (especially pyrocatechin) besides phloroglucin.

Phenol (carbolic acid).—Used, together with hydrochloric acid, as a test for lignin. The best preparation of it is its solution in hydrochloric acid: this is prepared by dissolving carbolic acid in warm hydrochloric acid, adding, whilst the mixture is cooling, sufficient hydrochloric acid to dissolve any precipitate that may be formed. Lignified cells, treated with this mixture and exposed to sunlight, assume a bright green colour in consequence of the presence of coniferin.

Aniline sulphate and chloride.—These salts are also used as tests for lignin in cell-walls; the chloride is preferable. They may be used in solution either in water or alcohol, but the alcoholic solution gives the best results. The section is first treated with the solution and then with sulphuric or hydrochloric acid respectively, or better, the solution may be kept slightly acidulated by one or other of these acids: the lignified cell-walls assume a bright yellow colour.

VI.—**Mixtures.**

Schulze's Solution[1] **(Chlor. Zinc Iod.).**—Used as a test for cellulose; cellulose cell-walls turn blue when treated with this mixture; corky and lignified cell-walls turn yellow, protoplasm brown, and starch blue.

[1] Since there has been some uncertainty as to the exact name of the botanist who introduced these reagents, it may be here stated that they were first used by Professor Franz Schulze, of Rostock. See *Flora*, 1850, p. 643.

It is prepared by dissolving zinc in pure hydrochloric acid, and evaporating the solution, on a water bath, in the presence of metallic zinc until it has a syrupy consistence ; it is then saturated with potassium iodide, and then with iodine ; a few grains of iodine should be left in the liquid after it is poured off for use. It may also be prepared by dissolving 25 parts of pure fused zinc chloride and 8 parts of potassium iodide in 8½ parts of water, filtering through asbestos, and saturating with Iodine.

On adding Iodine to Schulze's solution till precipitation begins, a fluid is obtained which stains the cell-walls yellow, and the callus of sieve-plates a deep brown (Russow.)

Schulze's Macerating Fluid.[1]—One gramme of potassium chlorate is dissolved in 50 c.c. of nitric acid ; the tissue is then placed in a small quantity of it, and the whole is boiled for a short time in a test tube ; the liquid is poured off, and the residue is well washed with water. A filter may be used for washing.

The cells become isolated in consequence of the solution of the middle lamella.

[1] See Note, p. 22.

II.

The Structure and Properties of the Cell.

A.—*General Structure.*

Cut longitudinal sections of a parenchymatous tissue, the young shoot of the Elder, for example, mount in water, examine the parenchymatous cells of the pith with a high power ;

Note, 1, the **Cell-wall,** transparent, colourless, and apparently homogeneous ;

2, the **Protoplasm,** forming a layer (the primordial utricle), closely lining the cell-wall, and connected by bridles with a more centrally placed mass in which—

3, the **Nucleus,** a well-defined, roundish, highly refractive body, is situated ;

4, the **Vacuole,** filled with colourless fluid, the **Cell-sap.**

Structure of the Protoplasm and Nucleus.—Harden a small piece of a young growing shoot or root of *Pinus* in picric acid or in absolute alcohol ; stain with ammonia-hæmatoxylin ; mount in dilute glycerine, or stain with Kleinenberg's hæmatoxylin, and mount in Canada balsam ; examine with a high power :

Observe in the protoplasm—

1. The **Ectoplasm,** a hyaline layer, but little stained, next to the cell-wall.

2. The **Endoplasm,** the more internal, deeply stained protoplasm ; note that the staining is confined to fibrillæ which form a sort of network in the endoplasm, and to numerous minute particles, the **Microsomata.**

Observe in the nucleus—

1. Stained fibrillæ forming apparently a reticulum (**chromatin**).

2. The unstained matrix (**achromatin**) in which the fibrillæ are imbedded.

3. **Cell-division.**

In order to study the process thoroughly the hairs on the stamens of *Tradescantia* may be taken. A stamen is to be removed from a bud, on a warm day, and is to be placed at once in a drop of 1 per cent. sugar-solution on a cover-slip ; the cover-slip is then to be placed over a moist chamber as previously described. A magnifying power of about 500 diameters is to be used.

A terminal cell of one of the hairs, with a large and conspicuous nucleus, is to be observed. It will be seen that the nucleus gradually elongates in the direction of the longer axis of the cell ; it becomes more granular, and the protoplasm of the cell aggregates at its poles ; then the nucleus presents a striated appearance, the fibrillæ gradually arrange themselves parallel to the longer axis of the nucleus, and approach each other at the poles ; thus a characteristic nuclear spindle is produced ; the fibres are then ruptured in the equatorial plane, and gradually collect at each pole, so that two new nuclei are found. A layer of granular protoplasm (the cell-plate) consisting of microsomata, is now found in the equatorial plane, and it extends on each side until it reaches the wall of the cell ; this layer becomes converted into cellulose, and constitutes the dividing wall between the two cells.

Good preparations of nuclei may be obtained by making longitudinal sections of growing points (*e.g.* of the young roots of *Pinus*), and staining with hæmatoxylin.

4. Structure of Chlorophyll-corpuscles and of Leukoplastids.

a. Chlorophyll-corpuscles, or chloroplastids. Mount a thin leaf of a Moss (*e.g.* Funaria), or the prothallus of a Fern, in water ; note the corpuscles in the cells.

Treat with alcohol; the green colouring matter (**chlorophyll**) is gradually dissolved out, and the corpuscle is left colourless.

Press out the contents of an internodal cell of *Nitella* or of *Chara* on a slide; put on a cover-slip and examine with a high power. Run in some distilled water: observe that the corpuscles swell up, assuming the form of large hyaline vesicles; the chlorophyll is confined usually to one portion of the vesicle.

If chlorophyll-corpuscles, which have been treated with picric acid and decolorised with alcohol, be stained with iodine, Hoffmann's blue, or hæmatoxylin, and be examined with a very high power, it will be seen that they have a trabecular structure; it is from the interstices of the trabeculæ that the colouring-matter has been removed. The leaves of *Vallisneria* afford good material.

The same result may be obtained by prolonged treatment with dilute acid (hydrochloric acid mixed with water in the proportion of 1 : 4, is most effectual), or by exposure for one or more hours to steam (Pringsheim).

The minute structure of the corpuscles can be very readily made out in cells of the leaves of *Echeveria*.

If the plants used have been previously exposed to light, it will be observed that the chlorophyll-corpuscles contain granules. If a decolorised corpuscle be treated with iodine, the inclosed granules will turn blue, showing that they are starch-granules (see p. 33).

b. Leukoplastids. These are colourless protoplasmic corpuscles of various shape, which are to be found in the cells of those parts of plants which are not exposed to light, and in which starch is deposited.

The material must have been previously treated for a short time with picric acid, so as to prevent their swelling up and disappearing when they are mounted in water or in dilute glycerine.

The most suitable material is any tissue of which the cells contain but few starch-granules; the best is the tubers of the orchid *Phajus grandifolius*, (*Bletia Tankervilliæ*). In this starch-grains can be easily seen borne on the leukoplastids.

5. **Structure of Thickened Cell-walls and of Starch-grains.**

a. **Cell-walls.**—Cut a transverse section of an old branch of *Clematis Vitalba;* mount in water; examine with high power.

Observe the thick-walled cells of the pith; the wall appears to consist of a series of concentric layers; this is described as the **stratification** of the cell-wall.

Strip a piece of the bark from the branch, and remove with a needle some of the fibrous internal layer of the bark; mount in water, tease out with needles, and examine with a high power.

Observe the dark lines running in the wall of the fibre at an acute angle to the longer axis of the fibre; note that these lines run in different directions in different layers of the wall of the fibre; this may be seen by carefully focussing first the surface and then the deeper layers of the wall; these lines are described as constituting the **striation** of the cell-wall.

Observe the canals running transversely across the cell-walls. Some of the cells will present their upper walls (those nearest the observer): on these **pits**

will be seen, which are the terminations of canals like those seen in the sections of the longitudinal walls.

Pits can be readily seen, without making sections, in the leaves of some species of *Trichomanes*.

Cystoliths may be included here, since they are developed from the cell-wall.

Cut a transverse section of a leaf of *Ficus elastica*: mount in water; examine with a high power.

Observe the layer of large clear cells underlying the superficial layer of the epidermis of the upper surface of the leaf: here and there one of these cells is seen to contain a botryoidal body suspended by a stalk from the top of the cell; this is a **Cystolith**: it consists of a mass of cellulose developed as an outgrowth from the cell-wall, encrusted with calcium carbonate.

Run in a drop of acetic acid: observe that the cystolith becomes gradually transparent, and that an evolution of bubbles of gas is taking place from it.

When the calcium carbonate is all dissolved, a mass of cellulose will be seen to remain, presenting both striation (from above downwards) and stratification (parallel with its margin). Apply tests for cellulose (p. 29).

b. **Starch-grains.**—Scrape lightly with the blade of a knife the freshly cut surface of a piece of a potato; mount the scrapings in a drop of water; examine with a high power.

A number of somewhat ovoid bodies of various sizes will be seen; these are **Starch-grains.**

Near the pointed end of a well-developed grain will be seen a small. round, clear spot, the **hilum.**

On each side of the hilum a number of layers will be seen, constituting the stratification of the grain.

The layers near to the hilum are concentric with it, and are complete; the more external layers are excentric, and many of those between the hilum and the broad end of the grain will be seen to be incomplete; hence the layers are more numerous between the hilum and the broad end than between the hilum and the pointed end.

Here and there will be seen a **compound grain,** consisting of two small grains in contact by their broad ends, and invested by several layers common to both.

B.—The Micro-Chemistry of the Cell.

I. The CELL-WALL.

a. **Cellulose** cell-walls.

i. Coloured faintly yellow by iodine.

ii. Coloured violet on treatment with Schulze's solution (p. 22).

iii. Coloured blue on treatment with iodine and sulphuric acid.

In some cases the cell-wall turns blue when it is treated with iodine alone, a substance allied to starch being probably present (amyloid); instances of this are, the asci of Lichens, the bast in the stem of *Lycopodium* and in the root of *Ruscus*, the endosperm-cells of *Pæonia*, and the cells of the cotyledons of various Leguminous seeds.

In other cases the characteristic reactions are not given on treatment with Schulze's solution, or with iodine and sulphuric acid; instances of this occur in the tissues of young seedlings, of growing-points, of the cambium, and of Fungi. In the case of young tissues it suffices to treat them previously with hydrochloric acid or with solution of potash for a short time; they then give

the reactions mentioned above; the tissues of Fungi require a long treatment (three or four weeks) with potash. It appears that in these cases other substances are present which must be extracted from the cell-walls before the characteristic cellulose-reaction can be obtained.

iv. Dissolved by ammoniacal solution of cupric hydrate and by strong sulphuric acid.

v. Stained by solutions of carmine and of hæmatoxylin which contain a mordant, by methylene blue, and in various degrees by other aniline colours.

b. **Lignified** cell-walls—

i. Coloured yellow by iodine and Schulze's solution.

ii. Coloured deep brown by iodine and sulphuric acid.

iii. Coloured bright yellow when treated with solution of aniline chloride or sulphate, the colour being intensified by subsequent treatment with hydrochloric or sulphuric acid.

iv. Coloured red when treated with solution of phloroglucin (p. 21), and with strong hydrochloric acid.

v. Coloured green when exposed to light ($\frac{1}{2}$—1 min.), after treatment with carbolic and hydrochloric acids (p. 22).

vi. Swollen and slowly dissolved in strong sulphuric acid; dissolved slowly in concentrated chromic acid; soluble in Schulze's macerating fluid (p. 23).

When the lignification is not complete the cell-wall becomes disorganised and dissolves partially in strong sulphuric acid; this is due to the presence of a considerable proportion of cellulose. Lignified cell-walls give the characteristic cellulose-reactions after maceration in Schulze's fluid. The solubility of lignin in this fluid affords a means of isolating the cells of a woody tissue.

vii. Stained slightly or not at all by solutions of carmine and hæmatoxylin, but readily by aniline colours

c. **Cuticularised** cell-walls (including cork)—

i. Coloured yellow by iodine, by Schulze's solution, and by iodine and sulphuric acid.

ii. Coloured yellowish by concentrated solution of potash; on gradually warming (without boiling), it becomes bright yellow; on boiling, yellow drops of suberin escape.

iii. On treatment with Schulze's macerating fluid, the cuticularised cell-walls become conspicuous; on boiling, viscous drops (impure suberic acid) escape, which are soluble in hot alcohol, ether, benzol, chloroform, and dilute potash solution. Traces of cuticularisation may be detected by treating the tissue for a short time with Schulze's fluid without heating, and then with potash; the cuticularised cell-walls become conspicuous and turn yellow; the colour may be intensified by gently warming in potash.

iv. Dissolved very slowly in concentrated chromic acid; hence on treatment of a section with this reagent the cuticularised cells are the last to disappear.

v. Not stained by solutions of carmine or hæmatoxylin; stained by aniline solutions.

The cuticle may be isolated, from the surface of a leaf for instance, by boiling for a few minutes in hydrochloric acid, and then washing with water.

d. **Callus.** To be found on the plates of the sieve-tubes.

i. Soluble in sulphuric acid.

ii. Stained by Hoffmann's blue, and by hæmatoxylin. The most delicate reagent for callus is the following: to a quantity of chlor. zinc. iod., add an equal volume of the ordinary solution of iodine in potassium iodide; to

the mixture add a saturated solution of iodine potassium iodide drop by drop, until precipitation begins. This mixture stains the callus a deep brown.

e. **Mucilaginous** cell-walls.

Resemble cellulose cell-walls in their reactions. On treatment with iodine and sulphuric acid they sometimes assume a brownish colour in addition to the blue.

Cell-walls which have become converted into gum do not turn blue on treatment with iodine and sulphuric acid: Hanstein's aniline-violet colours them red. Both mucilaginous and gummy cell-walls are stained by methylene blue.

Mucilages stain pink with corallin solution; certain kinds stain with Hoffmann's blue.

Gums stain with neither of these reagents.

f. **Mineral deposits** in cell-walls.

i. Silica.

On heating a section of tissue containing silica on platinum foil with nitric acid, a complete skeleton of the silicified cell-walls remains.

(ii.) Calcium oxalate.

Occurs in the form of crystals: insoluble in acetic acid; soluble, without evolution of gas, in nitric acid.

(iii.) Calcium carbonate.

Occurs either in distinct crystals, or, apparently, as granules: soluble in acetic acid with evolution of bubbles of gas (CO_2).

The most characteristic form in which it appears is in special outgrowths of the cell-wall which are incrusted with it; these are termed **cystoliths** (see p. 28).

II. The Cell Contents.

a. The **Protoplasm.**

i. Coloured yellow by iodine, and by Schulze's solution.

ii. Coloured yellow by nitric acid, the colour becoming more intense on warming; on the addition of potash or ammonia a bright yellow colour is produced (xantho-proteic reaction).

iii. Coloured violet after treatment with dilute solution of copper sulphate on the addition of potash. Fehling's solution may be used. See page 20.

iv. Coloured pink after treatment with syrup on the addition of dilute sulphuric acid.

v. Stains readily with solutions of carmine, hæmatoxylin, and Hoffmann's blue; bright red with Hanstein's aniline violet.

These reactions are given by all bodies consisting of proteids.

b. The **Chlorophyll-corpuscles.**

On treatment with alcohol the green colouring-matter (chlorophyll) is dissolved, and the substance of the corpuscle is left: this gives the reactions enumerated above as being characteristic of proteids.

The orange colour of many fruits and flowers is due to the presence of coloured granules which appear to be modified chlorophyll-corpuscles (chromoplastids). These may be well observed in the petals of *Tropæolum.*

c. The **Starch-grains.**

Coloured blue on treatment with iodine.

Coloured pink with corallin solution (p. 12).

In order to detect the presence of minute starch-grains in chlorophyll-corpuscles, the tissue must be kept in alcohol exposed

to light until the whole of the chlorophyll is dissolved out; it must then be treated for several hours in strong solution of potash; after neutralisation with acetic acid the tissue may be treated with iodine.

d. **Oil-drops.**

i. Coloured black on treatment with osmic acid.

ii. Soluble in alcohol, in ether, and in potash (saponified).

e. **Mineral substances.**

i. Calcium oxalate: occurs with two molecules of water of crystallisation in crystals belonging to the clinorhombic system (including raphides), or with six molecules in crystals belonging to the quadratic system. Clusters of crystals and sphæro-crystals may consist of crystals belonging to either system. Insoluble in acetic acid; soluble in nitric acid, without evolution of gas.

ii. Calcium carbonate: occurs usually in small crystals, the crystalline nature of which can only be ascertained by means of the polariscope. Soluble in acetic acid, with evolution of bubbles of gas (CO_2).

iii. Calcium phosphate (also magnesium phosphate): occurs in the form of granules (*e.g.* the globoids). Soluble in acetic acid without evolution of gas.

iv. Calcium sulphate: occurs in the crystalline form. Soluble with difficulty in acetic or nitric acid.

f. **Crystalloids:** may be seen in the more external cells of potato-tubers, in the form of cubes.

i. They give the reactions characteristic of proteids.

ii. Soluble in potash.

iii. Soluble in saturated solution of common salt.

g. **Aleurone-grains:** occur most prominently in oily seeds.

i. Give the reactions characteristic of proteids.

ii. Soluble, usually, in potash.

The reactions of these bodies are very different in different seeds; the following will serve as types:—

1. *Grains without crystalloids.*

(*a*). Soluble in water: peony, almond, cherry, apple.

(*b*). Partially soluble in water; more or less readily soluble in 10 per cent. solution of common salt.

α. Soluble in saturated solution of common salt: lupine, pea, bean, scarlet runner.

β. Soluble in saturated solution of common salt only after treatment with alcohol: sunflower, turnip, cress.

2. *Grains containing crystalloids.*

(*a*). Partially soluble in water; more or less readily soluble in 10 per cent. solution of common salt.

α. Soluble in saturated solution of common salt: Brazil nut, pumpkin.

β. Soluble in saturated solution of common salt only after treatment with alcohol: castor-oil plant, walnut.

In all cases a mass (globoid) of mineral matter remains behind after the solution of the grain; this is soluble in acetic acid. The sections should be examined in alcohol.

h. **Tannin:** gives the cells in which it is present a brownish colour.

i. Coloured deep brown by potassium bichromate, or chromic acid.

ii. Coloured greenish-blue by dilute solution of iron sulphate.

iii. On treatment with a solution of ammonium molybdate in a strong solution of ammonium chloride, either a voluminous yellow precipitate is formed (showing presence of tannin), or a red colour is produced (showing presence of tannic, *i.e.*, digallic acid).

i. **Resin:** occurs in drops in the cells bounding resin-passages as well as in the passages themselves.

i. Coloured red by tincture of alkanet.

ii. Coloured blue by Hanstein's aniline violet

iii. Decomposed by potash.

iv. Soluble in alcohol and ether.

k. **Caoutchouc:** occurs in the laticiferous vessels in the form of granules of different size in different plants: stains red with alkannin solution. By means of this reaction good preparations of laticiferous vessels can be made.

l. The **Cell-sap** may contain in solution:

1. Colouring matters.

2. Cane-sugar (as in the Beet-root) ($C_{12} H_{22} O_{11}$), which does not give a reaction with Fehling's solution. See p. 20. If much is present it may be made to crystallise out by treatment with absolute alcohol.

3. Grape-sugar (Glucose) ($C_6 H_{12} O_6$).

If a section be boiled in dilute Fehling's solution, it will, if the cells contain glucose, turn yellow, owing to the reduction of the copper. See p. 20.

The precipitate (cuprous oxide) appears in the cells under the microscope as small black granules.

4. Inulin ($C_6 H_{10} O_5$).

When the material or the section has been treated with alcohol, the inulin is precipitated in the form of sphæro-crystals, which may be readily observed. These crystals are insoluble in cold, but readily soluble in warm water, and in dilute acids and alkalies.

Coloured slightly brown by iodine.

5. Asparagin ($C_4 H_8 N_2 O_3$).

When a section of a tissue containing asparagin is treated with absolute alcohol for some time, the asparagin is precipitated in the form of prismatic crystals,

either in the cells or at their edge, which are readily soluble in water. The best method is to maintain a stream of alcohol under the cover-slip by means of blotting-paper.

A saturated solution of asparagin may be used as a further test; the precipitated crystals will not dissolve in it, but will be dissolved on the addition of water.

In performing these tests it is better to use longitudinal than transverse sections.

C.—*The Micro-physics of the Cell.*

I. **Imbibition.**

This term is used to express the fact that the cell-wall and certain of the cell-contents (protoplasm, starch-grains, aleurone-grains, crystalloids) usually contain a certain amount of water, termed the **water of imbibition.** The amount of water of imbibition may be made to vary by appropriate re-agents, and this involves variation in size of the body observed. These phenomena are best seen in cell-walls and in starch-grains; the cell-walls should be such as are thickened, and consist of cellulose; those which are chemically altered (either cuticularised or lignified) cannot be made to vary to any considerable extent.

Cut a transverse section of the petiole of the Sunflower (Elder or Mallow will do as well); mount in water; examine with high power.

Observe just beneath the epidermis, several layers of cortical cells, the walls of which are thickened at their point of junction (collenchyma).

Run in some potash solution, or some moderately

strong sulphuric acid; notice the swelling-up of the thickened cell-walls.

The swelling-up of starch-grains may be observed in the same way.

The amount of the swelling-up may be estimated by using a micrometer-eye-piece.

The thickened cell-walls of pith-cells of *Clematis Vitalba*, or those of seeds (Lupine, Date) are also suitable material for this purpose.

II. **Osmotic Properties.**

These can be most easily studied in cells which have coloured cell-sap.

Cut a rather thick section of a piece of fresh beet-root, and mount in water;

Observe, the thin **cell-wall;**

„ the layer of protoplasm (**primordial utricle**) which lines the cell-wall;

„ the red **cell-sap** filling the cavity of the cell (**vacuole**).

Note that the red sap does not escape from uninjured cells.

Examine a similar section which has been dipped for a moment into alcohol; the red sap diffuses out of the cells.

Hence it is evident that the colouring-matter cannot diffuse out of a living cell, but diffuses readily out of a dead cell.

Mount another section in water, and run some 10 per cent. Na Cl solution under the cover-slip; it will be seen that the red sap collects as rounded deeply-coloured bodies in the centre of the cells. This is due to the contraction of the primordial utricle.

A cell in this state is said to be **plasmolytic.** The contraction is due to the withdrawal of water from the cell-sap by the strong salt solution, this withdrawal not being compensated for by the entrance of salt solution into the vacuole. The salt solution diffuses through the cell-wall, and occupies the space between the cell-wall and the contracted primordial utricle, but it cannot pass through the primordial utricle to any considerable extent.

On washing the section with water, the plasmolytic cells gradually reassume their normal appearance.

From these observations it is evident that the passage of substances in solution into or out of the vacuole is controlled by the primordial utricle so long as the cell is living.

Plasmolysis can also be well demonstrated on a Fern-prothallus by treating it as above with salt solution; it will be seen that the contracted primordial utricle is connected to the cell-wall by a great number of delicate protoplasmic filaments.

III. **Optical Properties.**

1. Double Refraction.

In order to study this subject, apparatus for polarising light must be adapted to the microscope. This consists of two Nicol's prisms, one of which is fitted into an eye-piece, the other being fixed below the stage of the microscope, so that the light which is reflected from the mirror must pass through it: the former prism is termed the **analyser,** the latter the **polariser.**

The sections to be examined may be mounted in water or in glycerine, but the best results are obtained with sections mounted in Canada Balsam. A twig of a tree affords good material for observation. A thin, nearly median, longitudinal section is to be made and mounted: a high power must be used.

The examination is to be commenced by rotating the analyser, so that the field of the microscope is bright: the section will then appear much as it does when examined with an ordinary microscope.

The analyser is now to be rotated until the field is quite dark: it is then seen that the outlines of the cells appear bright, the thick, dense cell-walls (those of the fibres and vessels, for instance), being brighter than the thin cell-walls (those of parenchymatous cells).

This observation teaches that the cell-walls, but not the protoplasmic cell-contents or the cell-sap, are doubly refractive, and that the denser the cell-wall the more highly refractive it is.

A thin transverse section examined in the same way is seen to present similar appearances.

It will be observed, in addition, that the transverse section of a much thickened cell-wall (that of a bast-fibre, for instance), presents, when the field is dark, a dark cross: when the analyser is rotated through an angle of 90°, the dark cross is replaced by a bright one the field being also bright. For the explanation of this phenomenon reference should be made to textbooks of Physics.

Mount some starch-grains (potato) in water; examine as described above. It will be seen that when the field is dark the grain is bright and presents a well-marked dark cross; when the field is bright, the dark cross is replaced by a bright cross.

It will be observed that in examining sections in polarised light thick stratified cell-walls (particularly sclerenchymatous cells) are coloured; this is most apparent when the field is dark. This coloration is due to **interference** of light.

The phenomena of interference can be best studied by introducing a plate of selenite between the polariser and the analyser; it is to be placed on the stage of the microscope beneath the object. Various kinds of selenite-plates may be used; it is assumed here that the plate shows red and green tints.

Mount a section of a twig or of a leaf-stalk; rotate the analyser so that the field is red or green. The interference colours will not be well seen in the thin cell-walls; they will appear merely red or green. The thickened cell-walls will exhibit a play of colours which differs in different cases.

Mount a section of part of a succulent leaf (*Aloe*, *Crassula*, *Sedum*, &c.). Observe that the interference colours in the cuticularised external layer of the outer walls of the epidermal cells are complementary in position to those of the subjacent cellulose layers; this indicates differences of tension in the cuticularised and uncuticularised layers.

The relation of the interference colours can be more definitely made out in starch-grains.

Mount some starch-grains (potato) in water; rotate the analyser so that the field is red. Assuming that the starch-grain under examination is so placed that its long axis is directed away from the observer, it will be seen that there is a red cross on the grain corresponding in position to the dark cross mentioned above, that the two lateral segments of the grain are coloured yellow, and that the anterior and posterior segments are coloured blue.

2. Spectrum of Chlorophyll.

In order to observe this, an alcoholic solution must

be prepared. A quantity of fresh grass is to be taken and freed as far as possible from decayed leaves; it is then to be boiled in water, pressed so as to get rid of as much water as possible, and spread out on a sheet of paper to dry in a dark place; when dry it is to be put into a flask and alcohol is to be poured over it, and it is to be left for some hours in a dark place. When it is seen that the alcohol is coloured green, it is to be poured off and filtered; the solution is now ready for use.

The following is a convenient mode of examining the solution spectroscopically: The tube of a microscope is withdrawn (this may be easily done with the smaller forms of Zeiss', Hartnack's, and Crouch's microscopes), and it is replaced by a glass tube, the bottom of which covers the opening of the stage of the microscope; the sides of the tube must be made opaque by wrapping round them a sheet of black paper; the solution is then poured into the tube, and into the opening of the tube a microspectroscope is introduced; the mirror of the microscope is to be so inclined that it reflects a beam of light onto the bottom of the tube. The advantage of this method is, that it enables the observer to vary the thickness of the layer of the solution to be examined.

It is best to use a dilute alcoholic solution. Beginning with a column of the solution about $\frac{3}{4}$ of an inch in height, the spectrum will present a single rather narrow absorption band (band I.), in the red, about the line *C* of the solar spectrum, extending towards *B*; if the height of the column be about doubled, band I. will be seen to have become broader, a faint narrow band (band II.) will be seen to the right of it, between the

lines *C* and *D*, at the beginning of the orange, another faint narrow band (band IV.) in the green a little to the left of the line *E*, a broad faint band (band V.) in the blue to the right of the line *F*, a still broader faint band (band VI.) in the blue and indigo just to the left of the line *G*, and finally a broad faint band (band VII.) at the extreme violet end of the spectrum. On increasing the height of the column to about six inches, the bands I., II., IV. will be seen to have become broader and darker, and the bands V., VI., VII. to have coalesced so as completely to cut off the spectrum to the right of the line *F* in the blue; a new band (band III.) rather broad but faint, will be seen at the junction of the yellow and of the green a little to the right of the line *D*.

By this means it is possible to ascertain that the spectrum of chorophyll presents seven distinct absorption-bands.

PHANEROGAMÆ.

I. ANGIOSPERMS.

VEGETATIVE ORGANS.—(A) DICOTYLEDONS.

Embryo and Germination.

I. Examine the ripe fruit of the Sunflower (*Helianthus annuus*).

N.B.—The "seeds" sold for sowing are really achænia, including the products of development of both ovary and ovule.

It is a dry inferior achænium, with narrower basal, and broader apical end: at the latter is a scar, where were inserted the style and other floral organs.

Compare fruits *in situ* on the floral receptacle.

Dissect off the brittle **Pericarp**, from the anatropous and exalbuminous seed, which it incloses.

Note the delicate **Testa**, and, within this, the straight **Embryo**, of which the **Radicle** is directed towards the micropyle (*i.e.* towards the base of the fruit), and the two **Cotyledons** towards the apex of the fruit.

II. Compare plants, which have been germinated for different periods from one day to one week, and observe the following points in the process:—

1. The internal parts of the fruit swell, and cause the brittle pericarp to split longitudinally.

2. The radicle protrudes, and curves downwards.

3. The hypo-cotyledonary stem elongates, so that the pericarp and testa are carried upwards by the cotyledons, which remain inclosed by them for a considerable time.

4. The coats of the fruit fall from the cotyledons, which soon turn green, and expand as assimilating leaves, with the plumule seated between them.

5. The plumule develops leaves, which expand in succession.

6. The radicle has meanwhile elongated and produced lateral roots.

Notice that when the young root is removed from the soil, many particles adhere to it, especially at some distance from the apex; these are held by the **root-hairs** (*cf. infra*), which attach themselves closely to the particles of soil.

The internal changes accompanying the process of germination and more especially the redistribution of the reserve materials stored in the embryo, may be studied by cutting sections of the seedling at different stages of the process, and comparing the cell-contents in the corresponding tissues.

STEM—*HERBACEOUS TYPE.*

* Mature.

Observations with the Naked Eye.

I. Examine the whole of a well-grown plant of the Sunflower. The main axis or **Stem** is stout, herbaceous, and erect: it often develops to a considerable length without branching: it is cylindrical, slightly striated below, while the higher parts of it, where the

lateral branches are developed, are polygonal. Its surface is studded by stiff hairs, which are especially obvious on the lower portions of the internodes.

The stem bears laterally numerous **Leaves,** which are simple, petiolate,[1] cordate-acuminate, the margin slightly serrate, ciliated, venation palmate-reticulate, the surface hirsute. The arrangement of the leaves at the lower part of the plant (and including the **cotyledons** which wither at an early stage), is opposite, or in whorls of three; higher up this arrangement merges into the alternate, the complication increasing constantly upwards.

The stem is terminated by a **bud,** which may consist only of closely aggregated **foliage leaves** (or it may inclose the **reproductive organs,** which are contained in numerous flowers, closely aggregated so as to form a characteristic inflorescence—the capitulum, (*cf. infra*). Similar buds, in earlier stages of development, may be observed in the axils of the leaves (**axillary buds**).

Wash the roots and examine them. They are fibrous, and branch profusely. The primary (tap) root and earlier developed lateral roots are thicker than the later developed roots of a higher order (*cf.* secondary thickening of roots), the latter being successively thinner.

II. Cut the stem of a well-grown plant transversely at its thickest part, and smooth the surface with a razor.

The most prominent object in the section will be the massive, white, spongy **Pith** which occupies the centre.

[1] N.B.—The form of the leaves varies, the lower leaves of the plant being cordate, the upper ones lanceolate with winged petiole.

Around this will be seen, arranged more or less regularly in a circle, and near the periphery, a series of more solid-looking masses of tissue, these are the **Vascular Bundles.**

III. In order to obtain a clear idea of the course of these bundles, and of their connection with those of the leaves, cut off a piece of the stem, so as to include the insertion of a leaf or **node,** and about two or three inches of stem above and below that point. Bisect this longitudinally in a plane perpendicular to the median plane of the leaf. Clear away the pith with some blunt instrument, taking care not to injure the vascular bundles. This process will be made easier if the stem be boiled in water for about ten minutes.

Now dissect out carefully the course of the several vascular bundles, clearing away as much of the internal parenchyma as possible.

Treat the whole preparation with aniline sulphate and sulphuric acid for about five or ten minutes (*cf.* p. 22). The vascular bundles will be stained **yellow,** and their course may then be more readily followed. As in Dicotyledons generally, there are here no **cauline** but only **common** bundles (*cf.* Apex).

It will be apparent that in the internodes the bundles run **parallel to one another,** and as a rule without lateral fusion. This regularity is disturbed at the nodes (*a*) by **lateral fusions** of some of the bundles, but not of all of them, and (*b*) by the **entry of fresh bundles** from the leaves (usually three from each leaf), into the vascular ring.

IV. In a longer piece of the stem, follow carefully the course of several of the bundles entering from the

leaves, as far as they can be traced independently and without fusion. This will be possible at least for **one internode,** and usually for two or three; but the distance through which this independent course can be traced is variable in this plant. Further, the lateral fusions do not occur only at or near the nodes, instances may not unfrequently be found of fusions occurring at various points in the internodes.

That the arrangement and course of the vascular bundles in the dicotyledonous stem are connected with the arrangement of the leaves is an obvious fact. It may be seen in *Helianthus*, but is more prominently shown in plants with regularly decussate leaves (*cf. Cerastium*, *Clematis*, *Stachys*). Still the arrangement of the bundles may differ radically from that of the leaves, and is to a certain extent independent of them. This may be seen in such a case as that of *Iberis amara*, where the bundles do not run longitudinally, but in tangential spirals which have no direct relation to the arrangement of the leaves (Nægeli). The arrangement of the bundles in the normal dicotyledonous stem in a cylinder is due to the fact that each bundle as it enters from the leaf passes towards the centre of the stem for a certain distance only, which is approximately equal for all, each then curves gradually into a longitudinal direction. As regards the bundle-arrangement, *Helianthus* is not a very good type of an herbaceous Dicotyledon, still it illustrates the most essential points; *e.g.*, (1) the ring of vascular-bundles as seen in transverse section; (2) the entry of the bundles of the leaf-trace between the bundles connected with the higher leaves; (3) the lateral fusion of the several bundles at the node. Since the fusions often occur at points other than the nodes, and since the independent course of the bundles of the leaf-trace is of variable length it cannot be regarded as a perfect type. We therefore recommend a series of types for investigation, in which the vascular system has been carefully traced by Nægeli. In most of these it may be seen how closely the arrangement of the bundles is connected with (1) the arrangement of the leaves and (2) the number of bundles entering the stem from each leaf. *Iberis amara*, leaves alternate, leaf-trace with 1 bundle. *Lupinus*,

leaves alternate, leaf-trace with 3 bundles. *Cerastium*, leaves opposite, leaf-trace with 1 bundle. *Clematis*, leaves opposite, leaf-trace with 3 bundles. *Stachys*, leaves opposite, leaf-trace with 2 bundles.

The method which we have adopted in *Helianthus* is a coarse one, and only available in stout herbaceous Dicotyledons. When such a method is used we should always check our observations by comparisons of longitudinal sections of the apical bud (*cf. infra*) As a rule the subject should be studied in the first instance by making such longitudinal sections. These should be thick, and be cleared by treatment with dilute potash. Where the bud is not too bulky Nægeli adopted the method of bisecting the bud, clearing with potash, and drawing the bundle-arrangement in the two halves; hence the whole bundle-arrangement at the apex can be deduced from two such sections. As a further control, series of transverse sections should be cut through the apical bud; the order of these and their relative position must be accurately marked. A diligent comparison of these (with drawings) will supply the data for deducing the whole bundle-system. Finally, the results obtained by these two methods should coincide, if the observations be correct.

Microscopic Observation.

The material should be kept in spirit for some time to remove resin, and air, and to harden the tissues. This is not, however, indispensable, and fresh material may be used.

I. Cut transverse sections of a stem of a well-grown plant of *Helianthus, i.e.* of a stem more than half an inch at least in diameter.

Mount some of these in glycerine or glycerine jelly (these may be kept as permanent specimens), and others in Schulze's solution. Examine these first with a low power (1 in.), and observe the following tissues in succession starting from the exterior.

1. The **Epidermis,** a single peripheral layer of cells, not very well defined from the underlying tissues: it completely covers the surface.

N.B.—The margin is not perfectly regular, but is here and there extended outwards at the regions surrounding the bases of the large **multicellular hairs,** which may be recognised as being products of the epidermis.

Since these hairs are usually injured in cutting the sections, the width of their bases being greater than the thickness of a fine section, in order to see them well thick sections should be made specially, care being taken that the hairs shall not be previously injured before the sections are cut. They will then be seen to be long **conical hairs** with pointed ends, consisting of many cells, uniseriate: their bases are imbedded in cells of the epidermis and underlying tissue, which together form at that point a small **emergence,** on the apex of which the hair is borne. Other smaller hairs also occur. Compare the description of the apical bud (p. 64).

Beneath this single epidermal layer lies—

2. A band of tissue, several layers of cells thick, the walls of which are thickened at the angles where three or more cells meet, the cell-cavity being thus made oval or circular in transverse section; this is the chief characteristic of **Collenchyma,** of which this is a good type. Below this lies—

3. A band of thin-walled **Parenchyma,** in which are dotted here and there **resin-passages.**

Within these tissues of the **Cortex** (a general term including the tissues described under the headings 2 and 3) lie—

4. The **Vascular bundles,** which are wedge-shaped and are arranged in a ring: according to the stage of

development of the stem, and the point at which the section is taken, the bundles may be more or less completely joined laterally with one another. In old stems, and at or near the nodes this lateral fusion is most complete: still, under any circumstances the originally separate bundles can easily be recognised.

Centrally, *i.e.*, within the ring of vascular bundles is—

5. The **parenchymatous Pith,** consisting of thin-walled cells, which have for the most part lost their cell-nature (*i.e.* have no protoplasmic contents), and are filled with air: hence the whiteness of the fresh pith. (N.B. In material, which has been a long time in spirit, the air may have been removed by the alcohol, but this is usually a slow process.)

II. Choose out the thinnest of the sections, and examine it with a higher power (one-sixth inch or one-eighth inch), starting as before from the periphery of the stem.

1. The **Epidermal Layer** will be seen to consist of cells contiguous with one another, without intercellular spaces (excepting occasional stomata, which are, however, rare; *cf. infra*). The walls, and especially the external and internal walls, are thick, highly refractive, and show a stratified structure. In Schulze's solution they are blue (cellulose) with the exception of the outermost layer—the **cuticle**: this is a continuous, well-defined layer, which stains yellow, and may thus be easily recognised.

The granular protoplasmic contents of these cells (brown, Schulze's solution) are not plentiful, but form a thin layer lining the somewhat rounded cell-cavity.

Chlorophyll grains (*cf. infra*) may be found in them: this is an exceptional case, as they are usually absent from cells of the epidermis.

The cells surrounding the bases of the hairs are extended radially (as regards the stem), and the whole epidermis is at these points pushed outwards owing to luxuriant growth of the underlying tissue: in fact the hairs are each seated at the apex of an **emergence,** The nature of the hairs themselves will be studied later in connection with the apical bud.

2. In the **Collenchyma** the protoplasmic body resembles that of the epidermis: chlorophyll grains are numerous. The cell-walls also are highly refractive, and stain blue with Schulze's solution (cellulose): they are specially thickened at the angles, where three or more cells meet; in the thickened mass the **lines of stratification** are well seen. There is no sharp internal limit to the collenchyma, but it merges gradually into—

3. The thin-walled **Cortical parenchyma,** which differs from the preceding (*a*) in the thinness of its walls, (*b*) its less copious cell-contents, (*c*) the larger size of the cell-cavity.

Observe carefully the **resin-passages,** which occur in the cortical parenchyma. (N.B. The resin, being soluble in alcohol has been removed. To see it in its original condition sections may be cut from the fresh stem, and stained with tincture of alkanet.) They are **inter-cellular spaces,** formed by the splitting of cell-walls. The cavity thus formed is surrounded by small, thin-walled, **epithelium,** the cells of which divide both radially, and tangentially as regards the passage.

The development of the resin-passages may be observed with great ease and certainty in transverse sections of the stem of Ivy (*Hedera Helix*). Cut transverse sections from a young succulent stem, mount in glycerine. Scattered through the cortex and pith will be found passages already well developed, and having a structure similar to those in *Helianthus*. If the soft bast, which lies immediately outside the cambium, be examined carefully, resin-passages will be found in various stages of development, starting from a group of four cells, with no intercellular space. In older stages the cell-wall will be found to have split at the angle where the four cells meet, while in older stages again the intercellular space appears larger; meanwhile divisions (radial and tangential, the former more frequent) occur in the epithelial cells.

Note that in (1), (2), and (3), there occur, especially in stems growing apace, divisions of the cells in a radial direction. Compare the girth of the stem at the upper with that at the lower part of the plant, or that of a young plant with that of an old one. The conclusion will naturally be drawn that the stem increases in girth as it grows older, and since the outer tissues neither peel off, nor do the individual cells increase greatly in width, longitudinal radial divisions of the cells are the only alternative.

Before leaving the cortical tissue it must be noticed that the **Bundle-sheath,** which is the inmost layer of the cortical tissue, and which is easy of observation in the younger stem (*cf.* Hypocotyledonary stem) may be identified also in these sections, though with difficulty. The layer of thin-walled cells abutting directly on the thick-walled **sclerenchyma fibres** (yellow with Schulze's solution) show in their radial walls the characters of a bundle-sheath *i.e.* (i.), they are coloured brown with Schulze's solution; (ii.), they resist the

action of sulphuric acid; (iii.), they have the characteristic black dot (see p. 63). This layer may sometimes be traced as continuous round the ring of bundles, but this is difficult, owing to divisions in the cells of the bundle-sheath, similar to those above noticed in the **cortical tissue** and **epidermis.**

Treat some thin sections with sulphuric acid. The bundle-sheath and cuticle resist its action, and since they retain their sharp contour, they are thus brought into prominence.

Within this are—

(4.) The **Vascular bundles.** Select one of the largest of these for more minute examination: it will be found to consist of two well marked masses of thick-walled tissue (peripheral and central as regards the stem) with a transparent thin-walled portion between them. Further, on examining the latter more carefully it will be seen that the external part of it has thicker walls, and is less regularly arranged than the central portion, and must thus be distinguished from it. We have thus four portions of the bundle which, taking them in succession from the periphery to the centre, are named as follows:—

A. **Phloem.** { (i.) **Sclerenchyma.** (ii.) **Soft Bast.**

B. (iii.) **Cambium.**

C. (iv.) **Xylem.**

Taking first (*A*) the **Phloem** examine—

(i.) The **Sclerenchyma.** This appears as a half-moon shaped mass of tissue consisting of elements with rounded cavity, in which may be recognised the remnants of protoplasmic contents. The walls are

thick, and **lignified** (yellow with acidulated aniline sulphate, or with Schulze's solution). They also show differentiation into layers, of which the most prominent is the bright-looking **middle lamella.** Perpendicular to the internal surface of the walls may be seen **pits.**

(ii.) The **soft bast** consists of elements of very different structure and function: these are:—

(*a.*) **Sieve-tubes,** which appear in transverse section as the larger cavities of the soft bast: their walls are rather thin and consist of cellulose (blue, Schulze's solution). Occasionally these cavities will be found traversed by transverse septa, having a punctate appearance. These stain dark brown with iodine solution. They are transverse **sieve-plates.** (*Cf.* below, description of sieve-tubes in Cucurbita.)

(*b*). Abutting directly on the sieve-tubes, and appearing as though they had been cut off from the sieve-tube by a longitudinal wall, may be seen smaller cells. These are the **companion cells.**

(*c*). The remaining elements resemble the sieve-tubes in transverse section except in their smaller size, and absence of sieve-plates. These are **cambiform cells,** or **phloem parenchyma.**

Passing inwards, the distinction of these several constituents of the soft bast becomes more difficult, while the walls are thinner, and the arrangement of the elements is more regularly in radial rows, till, in the band of thin-walled tissue which borders immediately on the xylem, these characters become very obvious. This band is—

B. The **Cambium,** or active formative layer. Its

constituents are cells arranged in radial rows, with thin cellulose walls (blue Schulze's solution), and plentiful protoplasmic contents: the tangential walls are the thinnest, hence we may conclude that the most recent divisions have been in this direction, and have been repeated. Occasionally traces of recent radial division will be found, but this is less common. The form of the individual cells varies from oblong to square, as seen in transverse section: in the former case the longer axis is tangential. Trace the radial series outwards into the phloem, and inwards into the xylem: they may often be followed for a considerable distance with certainty. Note how, in passing from the cambium to the phloem or xylem the cells divide, and how the form of the individual cells is modified. Hence we may draw conclusions as to the development of the different tissue-elements of the mature xylem and phloem from the originally uniform cells of the cambium. For further details *cf.* the Elm and Pine, which, being lignified stems, and having more definite secondary increase, are better types for the study of cambium.

C. The **Xylem** also consists of elements of various structure: of these the most noticeable are—

a. The **Vessels**, easily recognised by their large cavity: they are arranged in radial rows, the individuals decreasing in size towards the central limit of the bundle. The walls are thick and lignified (yellow with Schulze's solution, or with H_2SO_4 and aniline sulphate), they have no protoplasmic contents; their further distinctive characters can only be seen in longitudinal sections. **Thyloses** may be observed, [*cf.*

infra, p. 61], especially in more central vessels. The vessels are embedded in a mass of tissue composed of two tissue-forms, which, however, are not readily distinguishable in transverse sections: they are—

b. **Xylem-**, or **wood-fibres,** which appear irregular and polygonal in transverse section, and have thick lignified walls: cell-contents not prominent, or absent.

c. **Xylem-parenchyma**—cells which retain their protoplasmic contents; their cell-walls are lignified, or of cellulose: the latter is the case with those cells which surround the more central vessels. This constituent of the bundle is often absent, and is not characteristically represented in this case (*cf.* stem of Elm, *infra*).

5. The **Pith** consists of cells, which have for the most part lost their cell-contents: they are very thin-walled; the walls are slightly pitted: intercellular spaces small. The cell-cavity is usually filled with air, which replaces the protoplasm, especially near the centre; hence the whiteness of the pith.

III. Cut radial longitudinal sections of an old stem of *Helianthus*, and choosing such as have passed through a vascular bundle (easily recognised with the naked eye), treat them as above.

Bear in mind the observations already made on the transverse sections, and compare those results with the observations about to be made.

To complete the study of the tissues it would be necessary also to cut tangential sections, and, in the case of tissues in which the radial differ from the tangential walls, such sections must be made, and the comparison drawn between them and the transverse and radial sections (*cf.* stem of *Pinus*). In the present case, however,

this is hardly necessary, since the components of the several tissues of this stem appear almost uniform in their tangential and radial aspects.

Starting as before from the periphery, note successively the following tissues[1]:—

1. The **Epidermis**, consisting of oblong cells, whose walls and contents present the appearance already observed in the transverse sections. Note the disturbance of their normal arrangement around the bases of the larger **hairs**.

Beneath the epidermis lies—

2. **Collenchyma**, consisting of oblong cells with thick longitudinal cellulose walls (blue, Schulze's solution), and thin transverse ends : the contents are protoplasm, with a **nucleus** and **chlorophyll-grains**. Below each of the larger hairs the collenchyma gives place to short, thin-walled parenchyma, which, together with the epidermis covering it, forms those **emergences** on the summit of which the hair is seated. Within this is—

3. Thin-walled **Cortical parenchyma**, the cells of which are shorter, but wider, than those of the collenchyma ; there is however no sharp limit between them : observe transitional forms. Cell-contents resemble those of (2), but there is less chlorophyll.

Note the **resin-passages**, the course of which is directly longitudinal; they therefore appear as longi-

[1] It is but rarely possible to see all the tissues here enumerated satisfactorily represented in a single radial section, therefore the study of the tissues and their relative positions should be conducted by comparison of a number of sections one with another.

tudinal bands of small, oblong, thin-walled cells (**epithelium**).

The **Bundle-sheath** may occasionally be recognised as the layer of cells immediately outside the bundle. Very commonly starch grains may be detected in its cells.

4. The **Vascular bundle.** Supposing the section to have been approximately median through the bundle, the following components will be found to be included in it:—

A. **Phloem**, which is made up of—

i. **Hard Bast, Sclerenchyma,** or bast fibres. These appear in longitudinal section as long **prosenchymatous** cells, occasionally divided by more or less oblique septa. Walls thick, lignified (yellow with Schulze's solution, or with acidulated aniline sulphate), and pitted: remnants of the protoplasmic contents may be found, especially if the stem cut be not very old.

ii. **Soft bast**, consisting of tissues with cellulose walls (blue with Schulze's solution) and abundant protoplasmic contents: its several constituents are—

a. **Sieve-tubes**, long tubular structures with thin walls and transverse or oblique septa (**sieve-plates**), the structure of which is the chief characteristic of the sieve-tubes; they are readily recognised in sections treated with Schulze's solution (or iodine solution) by the deep brown coloration of the protoplasm, which is collected round the sieve-plates.

Treat some sections with potash: the protoplasm, and mass of **callus** surrounding the sieve-plates, swells, and the perforated or sieve-like character of the septum, which does not swell, is then easily recognised. Sieve-plates occur occasionally on the lateral walls,

where two sieve-tubes are contiguous. The sieve-tubes will be more easily recognised in sections which have been stained with Eosin (see p. 12).

For more accurate study of these structures, see sieve-tubes of *Cucurbita* (p. 84).

b. Side by side with the sieve-tubes may be found the **Companion cells** which are smaller sister-cells of the segments of the sieve-tubes, cut off during development: these are, however, difficult to distinguish, but their presence is proved by the transverse sections.

c. **Bast-parenchyma,** or **Cambiform cells.** These are oblong parenchymatous cells with thin cellulose walls (pitted, but not very distinctly) and protoplasmic contents.

B. The **Cambium,** a band (here very narrow) of oblong cells with very thin walls, and dense protoplasmic contents. As the tissue in this case differs in no essential point from that in other plants treated elsewhere, and as it is here difficult to study, its description will be deferred, though its presence here must not be forgotten.

C. The **Xylem,** consisting of—

a. **Vessels,** which are its most prominent constituent. They are structures with lignified walls (note reactions), which are variously marked; they have no protoplasmic contents, their wide cavity containing water or air. The cavity is continuous, owing to the partial or complete absorption of the transverse or oblique septa. Note instances of this partial or complete absorption. According to the various markings, or thickenings, of their walls, the vessels may be grouped under the following heads, the first named being the nearest to the periphery of the stem:—

(*a.*) **Pitted vessels,** which are the largest, having very large cavity, walls with **pits** which appear oval in surface view, and which have the same characters as the round bordered pits of *Pinus.*

Having observed the pits in surface view, focus so as to obtain a longitudinal optical section of one of the walls (or better, find a place where the preparation is so thin as to show this in real section). Compare this with what was seen in surface view.

(*β.*) **Spiral vessels** found in the more central part of the xylem, those most central having the spirals more closely coiled. Note transitional forms (irregularly **reticulated**) between spiral and pitted vessels.

(γ.) **Annular vessels** found at the central limit of the xylem, the thickening is here in the form of rings; in mature stems these vessels are usually more or less disorganised.

b. **Fibrous cells** (wood fibres), which are long and pointed: it is difficult to follow one individual fibre throughout its whole length, owing to its taking a sinuous course, the fibres being interwoven one with another: their walls are lignified and pitted: the cell contents are reduced or absent.

c. **Parenchyma,** which is to be found more especially around the vessels near the central limit of the bundle. The phenomenon of **thyloses** is the result of the encroachment of these cells on the cavity of the vessels. The normal individual cells are oblong with square ends, they have cellulose walls (reactions), and retain their protoplasmic contents.

The cells termed **thyloses** (Tüllen) are properly included under the term xylem parenchyma, being derived directly from this

tissue in the following way. When fully developed the vessels have lost their protoplasmic contents and their turgescence; their walls are unevenly thickened, at some points being thin (= pits) at others strongly thickened. If thin-walled tissue, the elements of which are active and turgescent, abut on such a wall, it is obvious that but slight resistance to the internal tension will be offered at the pits, where the wall of the vessel is thin. As a result the wall bulges at these points, and the cells encroach as papillæ upon the cavity of the vessel. Cell-divisions may occur in these papillæ, and the whole process be continued till the cavity of the vessel is completely filled with a cellular tissue.

Look in the longitudinal sections of the old stem of *Helianthus* for instances of such encroachment of cells upon the cavity of the vessel. Good results may be obtained from the old stem, or root, of *Cucurbita*, and from the stems of *Robinia*, or *Vitis*.

5. The central **Pith** is composed of parenchymatous cells, with thin walls consisting of cellulose (reactions) slightly pitted: they have lost their protoplasmic cell-contents in many cases, and especially near the centre of the stem. Occasional **resin-passages** may be found in the pith.

* *Young Stem.

IV. Cut transverse sections of a young stem, *i.e.* not more than one-eighth of an inch in diameter.

If the sections be cut from the hypocotyledonary stem, though they will correspond in all important points to the following description, they will differ in some minor details; *e.g.* hairs will be absent, the bundle-sheath will be more obvious, &c.

Mount in glycerine, and passing from the periphery inwards observe successively under a low power—

1. The **Epidermis** as before a single layer, with

hairs of various complexity and shape (*cf.* apical bud). Beneath this—

2. **Cortical tissue,** which is more or less clearly differentiated into—

α. **Collenchyma.**

β. **Cortical Parenchyma.**

γ. **Resin-passages.**

δ. **Bundle-sheath.**

These severally hold the same position, and have the same characters, though less strongly developed, as were above observed in the older stem.

The **bundle-sheath** in the young stem is more easily recognised than in the older stem. It is a continuous layer of cells, whose radial walls have a characteristic **dark dot** on each radial wall, due to reflection of light from the peculiar sinuous waves of the central part of the radial walls. The oblique part of each wave acts as a reflector, so that the greater part of the light is diverted before it reaches the eye. Hence the origin of the dark dot. The bundle-sheath lies immediately outside the vascular bundles, curving slightly towards the centre of the stem in the spaces between the bundles. It is more prominent in the hypocotyledonary stem, and especially when this is young. The cells are then filled with starch, and the layer may be readily recognised in sections treated with iodine. Under ordinary circumstances it is brought into greater prominence by treatment of the sections with potash.

Within the bundle-sheath, and arranged in a ring, lie—

3. The **Vascular bundles,** which are wedge-shaped, of variable size, composed of similar elements to those described above in the older stem.

Note that, if the stem be young enough, the bundles are not joined laterally as in the older stem, but are separated from one another by broad bands of ground

tissue. In slightly older stems the cells of this tissue may be found actively dividing, by tangential and occasionally by radial walls. An **Interfascicular Cambium** is thus formed, and by the tissues derived from it the vascular ring, as seen in the older stem, is completed. Centrally lies—

4. The **Pith,** consisting of thin-walled cells, with sparing cell-contents. These, then, have not yet lost their cell-nature; compare the older stem where the protoplasmic contents are replaced by air.

Note on Interfascicular Cambium.—We have seen that in the Sunflower the bundles are quite separate in the young stem, being isolated by masses of quiescent ground tissue. Later, the cells of the latter tissue begin to divide actively as an **interfascicular cambium layer,** lying between the originally separate bundles. This interfascicular cambium joins the margins of the fascicular cambium, and a complete **cambial cylinder** is thus formed. But here in the Sunflower, as in most herbaceous annual plants, the interfascicular cambium is not very long active; the product of its activity being but a narrow band of secondary fascicular tissue: the identity of the original bundles can thus be recognised at a glance. In some stems (*Ranunculaceæ*) the interfascicular cambium is completely absent.

Compare this with the case of most ligneous perennial plants, *e.g.* Elm, Pine.

Apical Bud.

V. Take the apical bud of a young plant, or of a young lateral branch of the Sunflower, and cut longitudinal median sections: treat with potash, and mount

in glycerine: examine with low power, and then observe—

1. That the axis ends in a naked, broadly-conical **Apex** (*punctum vegetationis*), which is surrounded and enveloped by—

2. **Leaves:** these may be observed in various stages of development, the youngest being nearest to the apex (*i.e.* their order of development is thus **acropetal**); the surfaces of the older leaves are covered with—

3. **Hairs,** which are absent from the apical cone and the youngest leaves (*i.e.* the hairs are developed subsequently to the leaves themselves).

Note (with a higher power) that the apical cone itself consists of thin-walled cells with plentiful protoplasm, which are smaller than the cells of the mature tissues already studied, and are in a state of active division (*i.e.* are **meristematic**). The whole meristematic mass is differentiated into parts, which may be distinguished more or less clearly from one another, and their continuity may be traced with the several tissue-systems of the stem and leaves, of which in fact they are the **formative** layers. We may thus distinguish the following:—

1. The **Dermatogen,** as a single continuous layer of cells, which divide only in a direction perpendicular to the external surface of the organ (stem or leaf), which it covers completely: it is easily seen to be continuous with the **epidermis,** of which it is the formative layer. Within this is a solid mass of tissue, which looks for the most part dark, owing to its being permeated by intercellular spaces filled with air. It is traversed at a short distance from the external surface by transparent, longitudinal bands of—

2. **Procambium,** which is the formative tissue of the vascular bundles. Trace its continuity with these. Between the procambial bands and the dermatogen lies—

3. The formative tissue of the **Cortex,** which is (partially at least) characterised by dark-looking intercellular spaces.

4. Centrally lies a dark bulky cylinder, which is continuous with, and formative of, the **Pith.**

Observe carefully the mode of **origin of the leaves.** They appear at the periphery of the cone as protuberances of the dermatogen and the subjacent cells. As they increase in size their internal tissues become differentiated into (1) procambium, which is subsequently connected with that of the stem, and (2) tissue with intercellular spaces, which is continuous with the cortex. At the same time single cells of the dermatogen grow out, and divide, so as to form the conical multicellular hairs, which cover the surfaces of the leaves (*cf.* leaf-section). In the older leaves of the bud the development of the emergences around and below the bases of these hairs may be traced.

Note on passing back from the apex towards the more differentiated part of the stem a gradual increase in length of the cells, corresponding to the gradual **extension** of the internodes, while in the stem (internode) below the bud this is very marked. Observe also the various stages of the process of **vacuolisation** of the protoplasm.

In cases where the apical cone is broad, as in *Helianthus*, the tissues, with the exception of the dermatogen, are usually not sharply defined from one another at a point immediately below

the apex; but the various tissue-systems appear to originate from a common meristem. In some cases, however (especially water plants), the definition is more marked. As an instance may be cited the apex of *Hippuris* (*cf. infra*, p. 82).

Node.

VI. Cut moderately thick longitudinal sections through a **young node** of the Sunflower, so as to include the median plane of the leaf (or of both leaves if they be opposite, as they often are in the lower part of the plant). Treat with potash and glycerine, and warm for a few minutes [or better treat with very dilute potash for twenty-four hours or more].

Mount in glycerine, and examine with a low power.

The course of the vascular bundles, which appear dark, is easily followed through the more transparent parenchyma. Note—

1. The continuity of tissues of the stem and petiole; there is no definite boundary between these two parts.

2. That the bundles from the petiole pass into the stem, and, curving at first inwards, they soon assume a longitudinal course.

3. That no bundle of the upper internode lies in the same vertical plane as the bundle which enters from the petiole, *i.e.* the bundle from the petiole enters between two successive bundles of the vascular ring.

4. If axillary buds be present, note how their bundle-system is inserted on the bundles of the main axis, as well as on those entering from the petiole. Observe the large multicellular hairs seated on the apex of small emergences as before seen (p. 50).

STEM—*ARBOREOUS TYPE.*

I. Note the following external characters of a twig of Elm (*Ulmus campestris*) of the current year. It is cylindrical, hirsute, green or brown according to age, the latter colour being due to the formation of **cork** (*cf. infra*, p. 70). Small brown excrescences are scattered over its surface; these are **lenticels.** The arrangement of leaves is bilateral, phyllotaxis $\frac{1}{2}$, branching axillary.

II. Cut transverse sections of a twig of the current year; mount in glycerine, and examine with a low power. [Other sections may, for comparison, be treated with Schulze's solution, others again with aniline sulphate and sulphuric acid.]

Observe the general arrangement of tissues in concentric layers, which will be found to succeed one another in the following order, starting from the outside:—

1. **Epidermis:** a single layer of small cells: many of them have grown out, as conical **hairs,** perpendicular to the surface.

2. **Cork:** consisting of one or more layers of square cells: it will be more strongly developed in older twigs, while it is completely absent in very young twigs (for development *cf. infra*). Here and there a **lenticel** may have been cut through: in which case it will appear as a lateral extension of the band of cork.

3. **Cortical tissue:** parenchyma with chlorophyll, and cellulose walls, and intercellular spaces; here and there are large transparent cavities (mucilaginous cells).

4.[1] Thick-walled masses of **Sclerenchyma** (hard bast), which form an irregular broken ring (walls brownish-red with Schulze's solution).

5. **Soft bast:** a transparent tissue with cellulose walls, and plentiful protoplasm.

6. **Cambium:** a misty layer of thin-walled tissue with plentiful protoplasm: cells in radial rows.

7. **Xylem:** a broad band of thick-walled lignified tissue, with crenated inner margin; centrally lies—

8. The **Pith** or medulla: round-celled parenchyma, with thin pitted walls: mucilage cells here and there.

The crenated appearance of the inner margin of the xylem is due to the presence of the wedges of **primary xylem** (forming the so-called **medullary sheath**), separated from one another laterally by parenchymatous bands, which may be followed outwards in a radial direction through the whole thickness of the vascular ring: these are the **primary medullary rays:** other rays will also be seen following a similar course, but extending only part of the way from the cambium to the centre and periphery of the vascular ring: these are **secondary medullary rays.**

Compare with the vascular arrangement of *Helianthus.*

Cut transverse sections through the axis of a bud, or of a young twig, during the process of extension in spring; treat with potash, and mount in glycerine. In these sections the vascular system will be found to be much less developed, but even here the primary bundles will not be found to be as clearly distinct from one another as in the young stem of *Helianthus.* In ligneous Dicotyledons the interfascicular cambium begins to be active at an earlier period than in those which are herbaceous.

[1] 4, 5, 6, 7, together form the **vascular ring.**

Examine the several tissues, above enumerated, in detail with a high power:—

1. **Epidermis:** a single layer of cells, with the outer wall thickened and cuticularised (test with the usual reagents): **Stomata** will be found in a normal position in young twigs, in older ones they are found at the apices of the lenticels (*cf. infra.* p. 72). Note the form of the conical **hairs,** the walls of which are silicified.

To obtain proof of the latter fact, treat tangential sections of the surface of the stem with potassium chlorate and nitric acid; dry them with blotting paper and ignite on a cover slip, or platinum foil; mount the ash in water, and treat with nitric acid. Silicified walls will after this treatment present the same outline as they originally did. In this case complete skeletons of the conical hairs will be found.

2. The **Cork** (when present) lies immediately below the epidermis: it consists of cubical cells, with thin walls, and little or no cell-contents: they are arranged, in radial rows, without intercellular spaces. Select a thin part of the section for special study of these radial rows, and note in each the following succession of tissues, passing from without inwards:—

a. A series of **Cork cells** as above described: walls stained yellowish-brown with Schulze's solution (**Periderm**).

b. At least one cell with very small radial diameter, and with protoplasmic contents and thin cellulose walls —**Cork-cambium** or **Phellogen.**

c. Cells with thick cellulose walls, and protoplasmic contents with chlorophyll: no intercellular spaces: this is the **Phelloderm,** which is also derived from the cork-cambium.

Treat a thin section with concentrated sulphuric acid: the walls of all the tissues will swell, and gradually lose their sharpness of outline, with exception of the cuticularised outer wall of the **epidermis,** and the **cork.**

N.B.—The cork is sometimes developed to an extraordinary extent on the twigs of the Elm, so that it appears externally as thick radial plates of tissue.

By comparing sections of twigs of various ages, starting from such as have just escaped from the bud, the following facts may be established—

i. The cork-cambium appears in the layer of cortical cells immediately below the epidermis.

ii. These cells divide parallel to the surface of the stem.

iii. The result of successive divisions in this direction is the formation of secondary tissues, which develop externally as cork, internally as phelloderm.

iv. The true cork-cambium consists of only a single cell in each radial row, from which, by successive division, all these secondary tissues are derived (*cf.* cambium of vascular bundles).

v. The cells of the cork-cambium occasionally divide radially.

As stems grow older, layers of cork appear successively further and further from the external surface: not only the cortex but also the outer and older portions of the phloem are thus cut off from physiological connection with the inner tissue; the term **Bark** is applied to tissues thus cut off, together with the cork which forms the physiological boundary. As a good example of such successive layers of cork may be mentioned the stem of *Vitis.*

Examine points where a **lenticel** has been cut through, or make median sections through a lenticel.

Note that here the cork layer widens out laterally so as to form a hemispherical mass (semicircular in section), which is covered by the extended epidermis; if the section be median, there will usually be seen a

stoma at the apex of the lenticel: the whole mass of tissue consists of cells of a corky nature, with intercellular spaces.

By comparison of sections of twigs of various ages it may be seen that lenticels originate below the stomata, by divisions of the subjacent cortical tissue by walls both radial and tangential; secondary lenticels are also formed later; these appear at points independent of the stomata.

3. The **Cortical tissue** is a broad band consisting of parenchymatous cells, with intercellular spaces. According to their various characters they may be thus grouped:—

a. Ordinary **parenchyma cells,** with cellulose walls and protoplasmic contents, with nucleus, chlorophyll, and starch-granules. The two latter are not constant.

b. Cells (idioblasts) with large **crystals.**

c. Large cells whose **mucilaginous** walls almost or entirely obliterate the cell-cavity.

Note that the cells (*a*) are subject to radial division, and that the whole cortical tissue is tangentially extended, so as to keep pace with the increasing bulk of the internal tissues.

N.B.—No obvious bundle-sheath is present in this stem.

4. The **Sclerenchyma** consists of cells with walls so thickened that the cell-cavity is often obliterated; the walls are differentiated into two or more strata. Reactions with aniline sulphate, light yellow; with Schulze's solution; brownish red.

5. The **Soft bast** is, as in the Sunflower, composed of several different thin-walled tissue-elements, which

are, however, difficult to distinguish in transverse sections. They are:—

a. **Sieve-tubes,** which are nearly circular in section, and usually of larger cavity than the other constituents.

b. **Bast-parenchyma:** cells often arranged in more or less regular radial rows: certain of the cells differ from the rest in containing one or more crystals.

The nature of these several tissues will be more successfully studied in longitudinal sections.

6. The **Cambium** consists of thin-walled cells arranged, as in the Sunflower, in **radial rows,** which may often be traced outwards into the phloem, and inwards into the xylem: the cells have copious protoplasm, in which a nucleus may often be observed.

Note that the tangential walls are thinner than the radial walls; also that the radial diameter of the cells is less than the tangential. These facts, together with the arrangement of the cells in radial rows, point to a sequence of divisions, by walls parallel to one another, in a tangential direction. If careful comparisons of a number of different radial series be made, it will be found that the arrangement is such as would result from the action of Sanio's law of cambial division (compare *Pinus*, p. 141).

7. The **xylem** also consists of several different tissue-forms, all of which have **lignified walls** (*cf.* reactions). They are:—

a. **Vessels,** easily recognised by their large cavity, and by the absence of any protoplasmic body. They occur, singly or in groups, scattered through the xylem.

It may be found that the cavity of some of the vessels is filled with a cellular tissue. This is especially frequent in the part of the xylem-ring nearer to the centre. The name **thylose** is given to such cells (see above, p. 61).

b. **Xylem-fibres** or **Wood-prosenchyma,** elements with much smaller cavity, little or no protoplasm, and thick walls.

c. **Xylem-parenchyma,** recognised by the presence of a protoplasmic body, and (at all events in autumn) of starch grains. The cells of this tissue are usually grouped round the vessels, and often form bands connecting two consecutive medullary rays laterally.

The cells of the **Medullary rays** are in the xylem thick-walled (lignified) and pitted; they have protoplasmic contents and starch. They are elongated radially. Note that they have special cambium cells, differing in form from the ordinary cambium. In the phloem the cells are thin-walled (cellulose), and have plentiful protoplasm.

8. The **Pith.** In the peripheral part the cells have thick, lignified, pitted walls, and a protoplasmic body with starch (at least in autumn). Tissue of this nature merges gradually into the central tissue with thin walls (lignified and pitted) and no protoplasm. Mucilage cells occur here and there.

III. Cut a four-year-old twig of Elm transversely, and smooth the cut surface with a razor.

Note, the age of a twig may be judged externally by counting backwards the annual increments of growth from the apex. The limits of each annual increment of growth may be recognised by the closer aggregation of the scars of the leaves or scales at those points.

Examine with a lens, and observe:—

1. The **Pith,** which occupies the organic centre of the stem. [Its position does not, as a rule, coincide

with the geometrical centre.] Externally to this lies:—

2. The **Xylem,** which is here a broad yellowish band, clearly marked off into a succession of concentric rings; these, as a rule, correspond in number to the years of the twig (**annual rings**).

3. The **Phloem,** which is a much narrower band than the xylem, is also marked off, though less distinctly, into concentric rings of equal number. Outside this lie:

4. The **Cortical tissue** and **Cork,** which are of insignificant bulk, compared with that of the vascular tissues.

Note the **medullary rays.** Some of these (**primary rays**) may be traced the whole distance from pith to cortex; others (**secondary rays**) only part of that distance. The latter have been entirely formed by the cambium.

IV. Cut transverse sections from the above cut surface, so as to include all the bands of tissue from the pith to the cortex: moisten them with alcohol, and mount in water or dilute glycerine. Examine with a low power.

Note that the constituents of the several tissues, produced during the later years, are similar to those already observed in the first year's stem; also that they are arranged, more or less regularly, in radial rows. This is best seen in the xylem: this points to their origin from the **cambium.**

Observe that the constituents of the autumn-formed xylem are smaller, and have slightly thicker walls than those formed earlier in the year, also that vessels of large cavity are absent from it. Hence arises the appearance of the **annual rings.**

V. Cut radial sections from a four-year-old stem of Elm; soak them for ten minutes or more in alcohol (to remove the air bubbles), and mount in glycerine. Use a low power.

It will be found difficult to cut good sections so as to include the whole radial surface; it is therefore better not to attempt it, but to study the several structures in a number of successive sections, each extending over only a part of the radial surface.

Starting from the outside, observe the same succession of tissues as already seen in the transverse sections, viz.:—

1. **Epidermis,** which is often dried up and disorganised.

2. **Cork** (including the **cork-cambium** and **periderm**), with the short cells arranged in radial rows.

3. **Cortical tissue,** with large **mucilage cells.**

4. **Hard bast,** consisting of long fibres.

5. **Soft bast,** thin-walled elements with much protoplasm.

6. **Cambium,** a misty band; cells not easily defined.

7. **Xylem,** with thick lignified walls, the vessels appearing as large tubular cavities.

8. **Pith,** parenchymatous; its appearance as in transverse sections.

Note the **medullary rays,** which appear as narrow bands of parenchyma, following the plane of section.

Examine these several tissues in detail with a high power.

1. The **Epidermis,** when still persistent, shows the same characters as are observed in transverse sections.

2. The **Cork** is composed of square cells arranged in radial rows, which are continuous through the **cork-cambium** to the **periderm,** the latter presenting much the same appearance as in transverse sections.

3. The **Cortical tissue,** which is parenchymatous throughout, also appears much the same as in transverse sections.

4. The **Hard bast** consists of long fibres, with thick walls, and very small cell-cavity: they are distributed in irregular groups among—

5. The **Soft bast,** characterised by thin walls and protoplasmic contents, and composed of—

a. **Sieve-tubes,** which are best seen in the part of the phloem nearest to the cambium. They resemble, in the main, those of *Cucurbita* (p. 84), but are not so wide; the **sieve-plates** are oblique, and face the radial planes. This is the usual arrangement of sieve-plates in secondary phloem; but their structure is often more complicated, *e.g.* in *Vitis, Tilia.* The sieve-tubes may easily be recognised in stems cut in autumn by the masses of **callus** which surround the sieve-plates: this stains brown with Schulze's solution. For the reactions of the callus, *see* p. 31. Companion cells are not easily seen.

b. **Bast-parenchyma:** oblong cells with cellulose walls, some contain protoplasm and starch. (More or less of the latter according to the season.) Others contain crystals: note the **medullary rays** as before.

Passing inwards the differentiation of tissues of the phloem is lost in—

6. The **Cambium,** which appears here as a narrow band of cells with thin walls, and abundant protoplasmic

contents. The form of the cambial cells may be better studied in tangential sections; here it is difficult to make it out.

7. In the **Xylem** (excluding for the present the medullary rays), observe the following structures, all of which have lignified walls—

(*a*). **Vessels** of various orders, which may be grouped as—

(i). **Spiral vessels** (**protoxylem**) found at the central part of the xylem, *i.e.* next the pith: they are usually more or less disorganised, being often filled with thyloses.

(ii). **Pitted vessels,** the lateral walls of which are crowded with bordered pits, of essentially the same structure as those in *Pinus* (p. 142). These vessels are usually of large cavity.

(iii). Vessels with both **pitted and reticulate** marking, superposed on one another on the same lateral walls: these vessels usually occur in groups, and are of small bore.

Note in all these, but especially in (iii.) points where transverse or oblique septa have been partially or completely absorbed.

(*b*). **Fibrous cells,** which occur in large groups, between the vessels: they are long, and prosenchymatous, and are intertwined, so that it is difficult to follow them through their whole length. Little or no cell-contents: walls not pitted.

(*c*). **Xylem-parenchyma:** oblong cells with protoplasmic contents, and starch: walls thick, lignified, and pitted: they occur in longitudinal bands: note their close contact on the one hand with medullary rays, on the other with vessels.

Examine the **medullary rays** in the xylem: they are composed of oblong cells, with their longer axes horizontal, arranged like bricks in a wall: in characters they resemble xylem parenchyma.

8. The **Pith** presents in radial section, for the most part, the same characters as already noted in transverse section.

VI. Treat some small pieces of the wood of the Elm with Schulze's macerating fluid (potassium chlorate, and nitric acid), and warm gently till the tissues break up, and the several constituents begin to separate: then wash with water, and mount in water or glycerine.

Some at least of the constituents will be found lying separately, or may be detached by slight pressure on the cover slip: the true form of the **wood-fibres** will now be seen. Note also **vessels,** and **xylem-parenchyma.**

VII. Cut tangential sections through the **xylem** of a 4–5 years' old stem of Elm, treat with solution of iodine, and mount.

Observe first with a low power—

1. The **Medullary rays** of lenticular appearance, easily recognised as masses of small thick-walled cells, filled with **starch,** which appears dark blue. (This is best seen in stems cut in autumn.) In close connection with these—

2. The **Xylem-parenchyma,** the cells of which also contain starch, and are thus easily recognised: note that it more or less completely surrounds—

3. The **Vessels,** the walls of which are stained yellow, and present those characters already observed in radial sections. The interspaces are filled by—

4. Masses of **Xylem-fibres,** which appear as before.

VIII. Cut tangential sections of the **phloem** of a similar stem : treat as before, and observe—

1. The form and arrangement of the **medullary rays** as in the xylem, but the walls of the cells are thinner, and not lignified : copious protoplasm is to be found.

2. **Phloem-parenchyma,** the cells of which differ in their cell-contents—

(*a*). Some containing crystals.

(*b*). Others with copious protoplasmic contents.

Both forms will be seen to have been derived by division from original elongated cells with pointed ends, since they are arranged in groups of this form. (*cf.* cambium.)

3. **Sieve-tubes** answering to the description given for radial sections (*cf. Cucurbita*). The sieves are oblique, the form of the successive segments oblong. The sieves are callous, and are easily recognised in sections stained with iodine or eosin.

4. **Bast-fibres** as before in radial sections.

IX. Cut tangential sections through the **cambium** of the stem of Elm : treat with dilute potash, and mount in glycerine. Examine first with a low power, and note that the general arrangement is similar to that already seen in tangential sections through the mature tissues, also that the form of the cells, in each part of the cambium-zone, is like or similar to the average form of the elements of the mature portion of wood or bast, which borders on it in a radial direction. Thus the cambium is differentiated into—

1. Cambium of medullary rays, which appears as

consisting of roundish cells, resembling cells of the medullary rays in form.

2. Cambium from which all the other tissues are derived, the cells of which have a prismatic form.

Taking these cells as a starting point, the several tissues above described are derived from them in the following way:—

(i). **Phloem.**—(*a*). **Sieve-tubes,** by lateral distension and conversion of the oblique walls into sieve-plates.
(*b*). **Parenchyma,** by division of the cells by transverse septa.
(*c*). **Fibres** (sclerenchyma), by elongation and interweaving of cells, the width of the cells at the same time being relatively reduced.

(ii). **Xylem.**—(*a*). **Vessels,** by lateral distension, and absorption of cell-contents, and of the terminal walls.
(*b*). **Parenchyma,** by division of the cells by transverse septa.
(*c*). **Fibres**, by elongation and interweaving of the cells, while the width of the individual cells is relatively reduced.

Observe intermediate stages between cambium cells, and these several mature tissues: this may best be done in sections cut from stems in early summer.

X. To investigate the nature of the **crystals,** several times observed in the parenchyma of the stem of

the Elm, cut tangential sections of the **phloem** or of the **cortical tissue,** mount in water, and having found one or more crystals—

(i). Run some iodine solution under the cover slip: the crystal is not stained.

(ii). Acetic acid: it is not attacked.

(iii). Dilute nitric acid: it is more or less completely dissolved.

These reactions, coupled with what is known from the analysis of ash, point to the conclusion that these are crystals of calcium oxalate.

STEM—*AQUATIC TYPE.*

Note the cylindrical smooth stem of the Mares-tail (*Hippuris vulgaris*), bearing whorls of simple leaves.

I. Cut transverse sections of an internode of the stem of *Hippuris vulgaris;* mount in glycerine and examine with a low power. Observe :—

1. A well-marked **Epidermis** with cuticle. Here and there are to be seen radiating scale-hairs. These occur especially in the axils of the leaves.

2. **Cortical parenchyma:** consisting of thin-walled, chlorophyll-containing cells, with large **intercellular spaces.**

3. A well-marked **Bundle-sheath,** with the usual characters, which immediately surrounds—

4. The central **Vascular Cylinder.** This is composed of :—

(*a*) A basis of thin-walled **parenchyma,** in which are distributed—

(*b*) In the central part **vessels** of the xylem with lignified walls,

(*c*) Towards the periphery elements with the characters of **soft bast;** the sieve quality is in this case doubtful.

II. Cut thick transverse sections of **nodes;** treat with potash, mount in glycerine; and observe, with a low power, that the

distribution of tissues is in the main the same as in the internode, but—

1. The large intercellular spaces are divided by horizontal septa, consisting of single layers of cells.

2. Branch bundles leave the central cylinder, and pass horizontally outwards to the bases of the leaves.

III. Cut median longitudinal sections of the apical bud of *Hippuris*, so as to pass through the elongated **apical cone**; treat with potash, and mount in dilute glycerine. Examine first with a low power, and observe :—

1. The **Axis**, which is wide, and cylindrical below, but tapers upwards to the rather elongated **apical cone** (*punctum vegetationis*). The axis is composed of the several tissues already noticed. Note especially :—

(*a*) The rectangular **intercellular spaces** divided transversely by septa at the nodes.

(*b*) The axial **vascular cylinder**, which may be followed far up into the apical cone, and which gives out lateral branches to the leaves.

2. The **leaves**, diminishing in size towards the apex. Note the **scale-hairs** about the bases of the leaves.

Put on a high power, and examine the apical cone. Note :—

1. The **Dermatogen**, (*cf.* p. 65) a continuous layer of cells, which covers the apical cone externally. Trace it backwards from the apex : it will be seen to give rise to the **epidermis**.

2. The **Periblem**, consisting of 4-5 layers of cells, which may be traced backwards, and be thus shown to give rise to the **cortex**.

3. A central cylinder of **Plerome**, which is continuous with, and gives rise to, the **vascular cylinder**.

Note that the **Leaves** originate from the outgrowth of the dermatogen and periblem, the plerome taking no part in their formation. Also that the vascular system of the stem is already developed at a higher point on the axis than that of any of the leaves. We have thus an instance of **cauline** vascular bundles, that is such as are proper to the stem, as distinguished from **common** vascular bundles, which terminate at their upper extremities in the leaves.

SIEVE-TUBES.

i. *Cucurbita.*

Though the sieve-tubes of the Sunflower are fairly large, the soft bast does not occur in large masses. In the Vegetable Marrow, however, the sieve-tubes are of extraordinary size, and occur in large numbers: this stem is thus excellently fitted for the study of the sieve-tubes of the type found in herbaceous stems.

I. Cut transverse sections of the stem of Vegetable Marrow, stain with eosin, and mount in water or glycerine.

The general arrangement of tissues in this stem differs in several important points from that in the Sunflower, and, indeed, from that in most herbaceous Dicotyledons. Thus :—

1. There occurs at a short distance below the epidermis a thick-walled band of **sclerenchyma** with lignified walls (yellow, with Schulze's solution, or aniline sulphate and H_2SO_4). This is quite distinct from the vascular bundles.

2. The vascular bundles are always separate and distinct: though an interfascicular cambium is formed in old stems, no secondary vascular tissue is derived from it.

3. The structure of the individual bundle is abnormal, there being in each bundle a central mass of xylem with the phloem masses lying, the one on the central, the other on the peripheral side of it. Between the xylem and the peripheral phloem mass is the cambium layer. The structure is the same in both phloem masses: either will therefore serve for the study of the sieve-tubes.

In the **soft bast,** which resembles that of *Helianthus,* but has larger constituents, observe—

(i). The transverse, circular, punctate **Sieve-plates,** having the same appearance as in *Helianthus,* and easily recognised by their contents being stained with eosin.

(ii). The **Companion-cells** appearing as though cut off from the side of a sieve-tube by a longitudinal wall.

(iii.) **Cambiform cells.**

Treat some sections with Schulze's solution; all the walls of the soft bast turn blue (cellulose), but the sieve-plates appear yellow or brown. (*cf.* longitudinal sections.)

II. Cut longitudinal sections through the soft bast: either radial or tangential sections will do. Mount some in iodine solution. The transverse **sieve-plates** will be brought into prominence by the deep yellowish brown staining of the mass of substance, which surrounds them: this may consist of—

a. A **Callus** mass, which immediately surrounds the plate, and is apparently a derivative of cellulose, though it differs from it in its properties: the size of the callus mass is variable according to season, age, &c., being greater in autumn, and in old sieve-tubes.

b. **Protoplasm,** which is usually collected in close contact with the sieve-plate (or callus if present), and more especially on its upper side.

Note, i. the **oblong form** of the segment of the sieve-tubes.

ii. The **companion**-cells, short, with granular protoplasm, and nucleus.

iii. **Cambiform cells** of similar form to the segments of the sieve-tubes.

Other sections should be stained with Eosin, then washed, and mounted in glycerine. The sieve-tubes will be readily seen as their contents will have stained deeply.

III. Mount some sections in water, and having found a sieve-plate with callus, run some dilute potash under the cover slip.

The callus mass swells: the protoplasm also swells: the section thus becomes more transparent, and the **cellulose basis** or true **sieve** becomes more apparent, and its pores can be easily seen. For further reactions of the callus, *see* p. 31.

IV. Treat some fresh sections with iodine, then dry off the superfluous fluid with blotting-paper, and mount in a single drop of strong sulphuric acid. The cellulose walls and callus will swell; the protoplasm will contract; look carefully over the protoplasmic contents of the sieve-tubes for the points where sieve-plates have been; here it will be found that fine strings of protoplasm, which passed through the sieve-plate, connect the protoplasmic masses on opposite sides of the sieve with one another (*cf.* Sachs' *Textbook*, Fig. 47.) By this reaction the **continuity of protoplasm** through the sieve is demonstrated.

It will be noted that the sieve-tubes of *Cucurbita* closely resemble those of *Helianthus*, the sieve-plates being transverse and simple. This is the usual type of sieve-tube to be found in **primary phloem** of Angiosperms, and generally in herbaceous stems of the same group. In the **secondary phloem** of ligneous stems a more complicated type of sieve-tube is found. This will be studied below in the stem of the Lime.

ii. *Tilia* (Lime).

I. Cut radial sections of the phloem of a stem of Lime more than three years old. Mount in glycerine and examine with a high power for **sieve-tubes**. The general arrangement of the phloem is similar to that in

the Elm. The sieves occur on oblique walls facing the radial plane, and are therefore here seen in surface view. Note that they have a similar appearance to those above described, but here three or more sieve-plates occur on each oblique wall.

II. Cut tangential sections of the same; mount as before. The oblique walls are here cut longitudinally; the sieve-plates are often **callous,** especially in autumn.

Note the form of the segments of sieve-tubes; it is fundamentally the same as that of the cambium cell, as seen in tangential section.

LATICIFEROUS TISSUES.

The material for the study of these tissues should be prepared by treatment with alcohol to coagulate the **latex.** Care should be taken to place the material in alcohol **directly it is cut,** or at least the cut surfaces should be wetted with alcohol so as to check the flow of latex from them. If the latex be allowed to escape, the laticiferous tissues are emptied, and are then much less easily traced than when they are full. The best method is perhaps to preserve the **whole plant** without injury in alcohol, in which case the latex will not be lost at all.

i. *Laticiferous Vessels.*

I. Cut tangential sections from the phloem of the root of the Dandelion (*Leontodon Taraxacum*), mount in potash and glycerine, and warm; examine under a low power.

The main constituents of the tissues are parenchymatous cells, with thin walls (**phloem-parenchyma**): **sieve-tubes** are to be met with here and there. The whole mass of tissue is permeated by a ramifying, and profusely anastomosing network of **laticiferous vessels.** The communication of these tubes with one another is demonstrated by the continuity of their contents (**latex**), which appear brown and granular.

The course of the vessels is mainly longitudinal, while lateral, horizontal branches frequently connect the parallel tubes.

With a high power make out more accurately the course of a group of the vessels.

II. Cut transverse sections of the same; mount in glycerine, and examine with a low power.

The laticiferous vessels appear circular in transverse section, and have brown contents; they are distributed in groups, which form more or less regular concentric rings round the central xylem.

Note in these sections the presence of **sphere crystals** of **Inulin.** In the former section they will have been dissolved by the treatment with potash. Observe that they are formed quite irrespective of the cell-walls, which are often included in them.

Treat the sections with iodine solution. They are not definitely stained.

Run some potash under the cover slip. They will be gradually dissolved without swelling.

The development of the laticiferous vessels may be traced by cutting thin longitudinal sections through the cambium of the root of the Dandelion. By careful comparison of such sections it

will be found that they originate from a number of originally separate cells of the cambium, the cavities of which are thrown together by the partial or complete absorption of the walls. Such fusions may appear in the terminal or the lateral walls.

ii. *Laticiferous Cells.*

I. Cut tangential sections of the cortex of *Euphorbia splendens* (other species will do) just outside the vascular ring, and mount in water, or dilute glycerine.

Examine with a low power.

Running through the cortical parenchyma will be seen long tubes, with thick cellulose walls and granular contents. These are the **laticiferous cells,** which differ from the preceding in being developed, not by fusion of originally distinct cells, but by continued apical growth of single cells.

Note cases of **branching** of these cells.

Included in the granular contents are **starch-grains** of peculiar dumb-bell form.

Treat sections with iodine solution, and observe the effect on these bodies.

II. Cut transverse sections of the same stem, and note the distribution of the laticiferous cells; they may be recognised by their walls, which are thicker than those of the surrounding tissues, and appear circular in section.

III. Separate the whole cortex from a piece of the stem; boil it in potash for about five minutes, and tease out the long laticiferous cells with needles; mount, and observe with a low power. They appear as long cylindrical structures, with thick walls (note striation). Observe occasional branching. They are usually broken at the ends.

LEAF.

A.—*PETIOLE.*

External Characters.—Note in the leaf of the Sunflower the channelled upper surface, and the insertion on the stem by a broad **Pulvinus;** in the axil may usually be observed an **axillary bud.**

I. Cut transverse sections of the petiole and mount in glycerine. The details of structure resemble in many respects those of the young stem, from which the petiole differs in the following points:—

1. The general outline of the section is semilunar, the concave being the superior (ventral), while the convex is the inferior (dorsal) surface : thus the petiole is **dorsiventral** whilst the stem is **polysymmetrical.** (This property extends also to the vascular bundles, of which the xylem is as a rule directed towards the upper surface.)

2. In the presence of numerous **Stomata** (two guard cells, *cf. infra*); beneath each stoma the collenchyma is replaced by chlorophyll-containing parenchyma with intercellular spaces. Note beneath each stoma the large **respiratory cavity.**

3. In the number and arrangement of the vascular bundles. In the petiole there are three main bundles, besides several smaller ones (*cf.* observation of stem with the naked eye, p. 47).

4. In absence of interfascicular cambium, the larger bundles are, for a time at least, **open** bundles, [*i.e.* have an active cambium,] while the smaller ones are **closed** [*i.e.* have no secondary thickening by cambium.].

5. No general bundle-sheath is present, though each bundle is surrounded by a layer of colourless cells without intercellular spaces, which may be regarded as representing the bundle-sheath.

B. — *LAMINA.*

Bifacial Type.

I. Take a piece of the lamina of the leaf of the Sunflower, including the apex: it is important that it should be previously bleached by treatment with alcohol; warm it gently in a mixture of dilute glycerine and potash, and mount in glycerine: examine with a low power, and observe—

1. The **midrib,** with its strongly marked **vascular bundle,** running up to the apex of the leaf, where it terminates abruptly in a mass of glandular parenchymatous tissue.

2. Lateral **branch-bundles** passing off from it, and forming a network by frequent anastomoses, while some branches run up into and terminate in the serrate projections of the margin of the lamina in a manner similar to the midrib as above described.

3. Smaller branch-bundles, which sometimes end blindly in the parenchyma filling the meshes of the network.

II. Cut off a small square piece of the lamina of a leaf of *Helianthus,* including one of the main **ribs** or **nerves,** and imbed in cocoa-butter or paraffin (*cf.* directions, p. 4), so that the rib shall be perpendicular. Cut transverse sections, and mount in glycerine. If cocoa-butter has been used, it may be dissolved off the sections with ether or chloroform.

Good sections may be obtained by holding the piece of lamina between slices of carrot, or pith ; or by folding the whole lamina repeatedly, and cutting sections from the whole mass. In these cases, though the chlorophyll appears of a better colour, the sections not having been treated with a solvent (alcohol), still the sections are infested with air bubbles, which may be partially removed by leaving the sections for some minutes in water ; they may be completely removed (though the chlorophyll would be dissolved) by treatment with alcohol. Difficulty will often be found in obtaining good preparations of the above ; all the important points may be more easily observed in the Cherry Laurel.

Note with a low power—

1. The general outline of the section, which is irregular and undulating, though it is in the main of uniform breadth. At the point corresponding to the main nerve the section widens out, the nerve appearing **semilunar,** as in the petiole. The convex side is the inferior (**dorsal**), and the concave the superior (**ventral**) surface.

2. That the margins of the sections (*i.e.* the superior and inferior surfaces of the leaf), are studded with projecting **multicellular hairs.**

3. That the arrangement of the tissues in the large nerve resembles that in the petiole, though less complicated. Thus it often has but one **large central bundle,** with smaller lateral ones. The position of the xylem and phloem relatively to the whole leaf corresponds to that in the petiole, *i.e.* xylem towards the upper surface, phloem towards the lower.

Occasionally some of the smaller bundles in the vein are inverted, showing an approach to the arrangement of bundles in the polysymmetrical stem.

4. **Smaller veins,** with correspondingly reduced

vascular bundles, are found scattered through the thinner part of the section.

Next examine the thinner part of the section, or the **Lamina** proper with a high power, and, starting the study of the several tissues from the upper surface. Note successively the following tissues:—

1. Upper layer of **Epidermis,** continuous with that covering the nerve; it is a single layer of cells, covered externally by **Cuticle,** and with the same characters as that of the stem (*cf.* p. 51). It bears numerous multicellular **hairs** (already studied in connection with the apical bud). **Stomata** occur in considerable numbers (*cf. infra*). Beneath this layer lie—

2. Thin-walled, oblong cells, with copious protoplasm, and **chlorophyll grains;** they are arranged with the longer axis perpendicular to the outer surface, and form two layers; this tissue, from the form and arrangement of the cells, is called the **Palisade parenchyma;** below it is—

3. A mass of parenchymatous cells of irregular form, with large intercellular spaces; in general characters they resemble (2); this is the **Spongy parenchyma.**

(2) and (3) are together included under the general term **Mesophyll.** Embedded between (2) and (3) are—

4. Numerous smaller **vascular bundles** (nerves) of various size, often reduced to a single pitted or spiral tracheide, surrounded by a colourless **sheath** of parenchyma similar to those in the petiole. The course of these bundles is diverse, since they form the reticulate system of veins; they may thus be seen in the sections

to have been cut transversely, obliquely, or longitudinally.

5. A second layer of **epidermis** bounds the section on the lower side; it has the same characters as the upper layer, but stomata are more frequent. Note the large **respiratory cavity,** and two small **guard cells.**

Hairs as before seen on the upper surface.

Note the mucilaginous walls of these hairs.

Since the leaf of *Helianthus* is not a universal type, it would be well to study also the structure of other types, for instance the coriaceous leaves of the Cherry Laurel (*Prunus Lauro-Cerasus*), and the cylindrical leaves of the Stonecrop (*Sedum acre*).

Special structural peculiarities are to be observed in the leaves of other plants; for instance, an epidermis consisting of more than a single layer of cells, *e.g.* in leaves of *Ficus*, *Piperaceæ*, *Begoniaceæ*, &c.; Cystoliths in the cells of the epidermis, *e.g.* *Ficus*, *Urtica*, &c.; glandular structures, *e.g.* in *Ruta*, *Psoralea*, &c., &c.

III. Taking that of the **Cherry Laurel**—sections may be prepared as above directed for the Sunflower, and be mounted in dilute glycerine. Starting from the upper surface, observe successively the following tissues—

1. **Epidermis,** a single even layer of cells, with thick walls, and colourless protoplasmic contents; no hairs or stomata are to be seen; the lateral walls are pitted; the outer wall is differentiated into—

a. **Cuticle,** a continuous, well-defined layer, covering the whole epidermis externally.

b. **Cuticularised layers,** of granular appearance; they are intermediate in properties between cuticle and true cellulose.

c. The **cellulose layer,** which abuts on the cavity of the cell.

i. These several layers may be readily distinguished in sections treated with fuchsin. *a* and *b* stain much more deeply than *c*.

ii. Treat sections with concentrated sulphuric acid. *a* retains a sharp contour; the rest of the wall swells, and loses distinctness of outline.

iii. Boil some sections for a long time with strong potash. *a* and the cuticular granules of *b* will be dissolved, while *c* and the cellulose matrix of *b* will remain.

2. The **Palisade parenchyma,** composed of thin-walled, oblong, closely-packed cells, with their longer axes perpendicular to the surface of the leaf; the cells are somewhat irregularly arranged in three layers; observe **nuclei** and **chlorophyll grains;** here and there are cells with but little protoplasm (**Idioblasts**) in which is inclosed a large crystal. Passing towards the lower surface of the leaf, this tissue merges gradually into—

3. The **Spongy parenchyma,** the cells of which resemble those of (2) in general characters; but their shape is various, and large intercellular spaces occur. Idioblasts with crystals are scattered here and there. Imbedded between (2) and (3) are—

4. **Vascular bundles** of various size; the direction in which these run is not uniform (*cf.* reticulate venation of leaf); the positions of xylem and phloem with regard to the whole leaf are the same as in the Sunflower; the bundles are surrounded by a continuous

colourless sheath of cells without intercellular spaces. At the lower limit of the section lies—

5. The lower **Epidermis,** which resembles (1) in general character; but differs in having numerous **Stomata.** Note the appearance presented where the two **Guard cells** of a stoma have been cut transversely, and observe carefully—

a. The form and position of the two guard cells.

b. The cavity or intercellular space between them (the **Pore**); this leads into—

c. The large, intercellular space (**Respiratory cavity**) in the tissue beneath the stoma.

d. In the sections stained with Schulze's solution or with fuchsin, note the continuity of the cuticle round the guard cells, into the pore of the stoma.

IV. Cut tangential sections from the upper and under surfaces of the leaf, and mount separately with the external surface in both cases uppermost.

The cells of the **upper epidermis** are tabular, with sinuous outline; the surface has a granular appearance (explained by the granular cuticularised layers observed in transverse sections); the lateral walls are pitted; contents colourless; **no stomata.**

The cells of the **lower epidermis** are similar to the above; but **stomata are numerous;** they have no definite arrangement. Note the two sausage-shaped nucleated guard cells, inclosing the pore; they contain chlorophyll.

(For development of stomata, *cf.* Hyacinth, p. 117.)

V. No **Subsidiary cells** are found in the Cherry Laurel. The leaves hitherto studied are of the **bifacial** type, *i.e.* the difference of the upper and lower

surfaces is recognisable by a different arrangement of the tissues at or beneath those surfaces. It may be noted that the leaf of the Cherry Laurel is of a more pronounced bifacial type than that of the Sunflower, since in the latter case stomata are found on both surfaces, while in the former they occur only on the lower surface.

Centric Type.

We have now to study leaves of the **centric** type, *i.e.* such as have their tissues arranged symmetrically. It is usually in succulent leaves that this arrangement is found, and they are of an approximately cylindrical form. As an example we may take the leaf of *Sedum acre* (the common Stonecrop).

VI. Cut transverse sections of the leaf of the Stonecrop; mount in water, or dilute glycerine, and observe that the **outline** of the section is even and oval; the arrangement of tissues is concentric, and is uniform all round, so that beginning at any point of the periphery and passing inwards we encounter—

1. The **Epidermis,** a single layer of cells of variable size and shape, with well-defined **Cuticle,** and **Stomata,** the guard cells of which are much smaller than the epidermal cells.

2. **Chlorophyll-containing mesophyll,** which is not differentiated into palisade, and spongy parenchyma; this tissue forms the great mass of the leaf; intercellular spaces occur; the cells are thin-walled, with a protoplasmic sac, in which are imbedded **chlorophyll grains,** and there is large central vacuole. Observe the chlorophyll grains undergoing division. Embedded in this tissue lie centrally—

3. **Vascular bundles** of small size: their number varies from 3 to 5.

Strip off a piece of **epidermis** from the leaf of *Sedum acre*, and mount in water. Note :—

1. The **Epidermal cells** with sinuous outline, nucleated: with no chlorophyll.

2. The **Stomata** with two guard cells surrounding the pore as in the Cherry Laurel. Surrounding these are :—

3. Three **Subsidiary cells,** which differ in size and shape from the ordinary epidermal cells, and are arranged in definite order round each stoma.

Beneath the epidermis will usually be found cells of the **mesophyll,** with thin walls, large vacuole and protoplasmic sac, in which are embedded **chlorophyll grains.**

By making similar preparations from successively younger leaves the development of the stoma and subsidiary cells may be traced as follows. From one of the similar epidermal cells a smaller cell is cut off, from this are successively cut off the three subsidiary cells, the remaining cell is the mother-cell of the stoma, which divides to form the two guard cells.

On the leaves of many plants, stomata of large size are to be found situated above the free endings of the vascular bundles of the lamina, and especially at the tips of the teeth: these are often incapable of closing, and are concerned in the secretion of water: hence they are called **water-stomata.** In certain cases (*Saxifragaceæ* and *Crassulaceæ*) a mass of cells of the mesophyll is specially differentiated as glandular tissue (the **water-gland**); it is connected with the termination of a vascular bundle.

ROOT.

Observations with the Naked Eye.

Germinate seeds of *Phaseolus multiflorus* (the Scarlet-Runner) in wet sawdust, or pure vegetable mould, till the primary root has attained a length of six to eight inches.

Note with the naked eye—

1. The **Seed,** from which the testa can easily be removed, disclosing—

2. The two fleshy **Cotyledons** (no endosperm is present) : between these—

3. The **Plumule,** which develops early as a stem, bearing foliage leaves.

4. Below the cotyledons a short **Hypocotyledonary Stem,** not clearly marked off externally, except by colour, from—

5. The **Primary root,** on the upper part of which are—

6. Numerous secondary, or **Lateral roots.** These are formed in acropetal order, and are arranged in regular longitudinal rows, usually four in number. On the youngest part of the primary root (*i.e.* within three inches or more of the apex) no lateral roots are to be seen.

Observe that particles of the sawdust, &c., adhere to the older parts of the roots, while the younger apical parts come out of the soil quite clean.

Microscopic Observations.

Harden the roots in alcohol for two or three days or more.

I. Cut transverse sections of the primary root at a point nearer the apex than the youngest lateral roots,

i.e. about two inches from the end. Treat with dilute potash for about ten minutes, and mount in glycerine.

N.B.—It will be found convenient to hold the roots in pith, or otherwise to imbed, while cutting the sections.

Observe the following tissues :—

1. At the centre of the circular section is a mass of Parenchymatous **Pith.** At the periphery of this are—

2. Four radiating groups of elements of the **Primary Xylem,** which are the most strongly marked tissues of the young root. They have dark lignified walls (test with Schulze's solution or aniline sulphate), and resemble the primary xylem of the stem. Note fresh elements in course of formation at their central limit. The development is thus centripetal. Alternating with these may be seen—

3. Four groups of **Primary Phloem,** which are not as yet very well marked. These several groups of elements are separated laterally from one another by bands of parenchyma. At the periphery of the central cylinder thus built up is—

4. The **Pericambium** or phloem-sheath, consisting of thin-walled cells, arranged in an undulating band, which is a single layer of cells in thickness, peripherally to the phloem, but opposite the xylem it consists of two to three layers of cells.

5. Immediately outside this is the **Bundle-sheath,** consisting of a single layer of cells, having the characteristic **dark dot** on their radial walls. Then follows—

6. The parenchymatous **Cortex,** a thick band of tissue, with intercellular spaces, and—

7. The **Epidermis,** a single layer, not well marked.

Single cells will be seen to have grown out perpendicularly to the surface as **root-hairs.**

II. Cut sections successively at older points in the same root, and observe the mode of origin of the **lateral roots,** noting more especially the following facts:—

a. The lateral roots arise opposite the groups of primary xylem: this explains their arrangement in four rows as above observed with the naked eye.

b. The pericambium, bundle-sheath, and a small portion of the cortex, all take part in their formation.

c. In the older lateral roots it may be seen that their vascular system is continuous with that of the main root.

This mode of origin of the lateral roots is the rule in the plants with apical meristem, arranged according to Type II. (*cf. infra*, p. 104). In the plants whose root-apex follows Type I. the lateral roots are mainly, or even entirely, derived from the pericambium.

III. Cut transverse sections of the root, six inches or more from the apex, taking care to avoid the lateral roots: treat as before.

The general arrangement of tissues is the same as has been above described, though there has been increase in bulk, and the xylem and phloem, being now more fully developed, are more easily recognised. Observe especially that the parenchyma, lying centrally to the phloem, has begun to divide repeatedly by tangential walls: in fact, four **cambium** bands are thus formed, from which is derived the **secondary thickening** of the root.

IV. Cut transverse sections of an old root of the Scarlet-Runner, and treat as before. Observe—

1. Centrally a parenchymatous **Pith,** relatively to which—

2. The four **Primary Xylem** groups retain their original position.

3. Four large wedges of **Secondary Xylem** have originated internally from the four cambium zones. These are separated from one another laterally by—

4. Four broad **Parenchymatous rays,** which lie on the same radii as the primary xylem. Outside the xylem is—

5. The **Cambium,** having similar characters to that of the stem, and giving rise peripherally to—

6. **Secondary Phloem.** Note if possible—

7. The four groups of **Primary Phloem** now separated from the primary xylem, but still on radii alternating with the latter. The section is bounded by—

8. **Cork** with a **Cork-cambium.**

Apex of the Root.

Type I. Cut thin median longitudinal sections of the apex of the radicle of the straight embryo of *Helianthus.*

[The arrangement of the meristem at the apex of the radicle of the embryo is similar to that of the apex of the growing root, and the former is chosen in this case as it is much easier to make preparations from it than from the growing root. The sections are of little use unless they are accurately median.]

Treat the sections with potash for ten minutes or more: wash with water, and mount in glycerine: examine with a low power, and observe that—

1. The mass of tissue is composed of thin-walled cells, arranged regularly in longitudinal rows.

2. That these rows of cells converge towards a point at some distance below the external apex of the root. This is the *punctum vegetationis.*

3. Note the **Procambium-cylinder,** or formative tissue of the vascular bundles, which pursues a longitudinal course up the centre of the root.

Examine with a high power: and observe that—

1. At some distance from the apex a definite layer of **Epidermis** covers the root externally. Follow this towards the apex: at some short distance from it this single layer splits into two: the inner is the **Dermatogen,** or formative layer of the epidermis: the outer is the outermost layer of the **Calyptra,** or root-cap. Following the dermatogen further inwards, it will be seen to split again several times in succession: the dermatogen may be traced as a continuous layer covering the inner tissues. The layers thus thrown off externally from the dermatogen form collectively the **Root-cap,** or **Calyptra.** We have in this case a common formative layer for epidermis and root-cap (*cf.* root of Maize, p. 120.).

2. Between the procambium and epidermis lies a broad band of formative tissue of the cortex, or **Periblem:** follow this to the *punctum vegetationis:* it is also a distinct continuous band, though reduced to a single layer of cells at the apex.

3. The **Plerome,** or central procambium cylinder, may also be traced as distinct up to the apical point.

This type of arrangement of tissues of the meristem may then be expressed thus:—

Calyptrogen } a single layer of cells, *i.e.* epidermis and
Dermatogen } root-cap have a common origin.

Periblem, distinct from the rest.

Plerome, distinct.

To this type belong most of the Dicotyledons. The work may be equally well done on *Linum usitatissimum*, or *Polygonum Fagopyrum*.

Type II. Prepare median longitudinal sections of the apex of the radicle of *Phaseolus multiflorus* (the Scarlet-Runner), and treat as the above. Examine with a low power and make out—

1. Calyptra (Root-cap).
2. Epidermis.
3. Periblem.
4. Plerome, forming the procambium and pith.

But here all the different tissue-systems will be found to originate from a general meristem, the original formative tissue of none of them being distinct from that of the others. This type may be expressed shortly, thus :—

Calyptrogen, Dermatogen, Periblem, Plerome } All united in a general, undifferentiated mass of meristem.

As alternative plants of the same type, may be named *Cucurbita* and *Pisum*.

VEGETATIVE ORGANS.—(B.) MONOCOTYLEDONS.

Embryo and Germination.

Soak fruits of the Maize (*Zea Mais*) in water for several hours.

N.B.—The fruit is a caryopsis, and results from the develop-

ment of both ovule and ovary ; its form is compressed conical, the apex of the cone being the basal point of attachment of the fruit.

I. Strip off the external coat of the fruit: this represents both the **wall of the ovary** and the **integument** of the ovule.

If sections be cut, these two layers may be distinguished from one another, under a low power.

Distinguish in the body of the fruit which remains—

1. A lateral, smaller, white portion: this is the **Embryo.**

2. A larger yellow part, which forms the greater mass of the fruit: this is the **Endosperm.**

Separate the embryo from the rest, and note its shape.

II. Cut longitudinal sections of the fruit, so as to include the axis of the embryo: mount in glycerine, and examine with a low power; observe—

i. The coat of the fruit, consisting of two layers. Note at the apex of the fruit the remnant of the **Style,** and at the base the attachment.

ii. The **Endosperm,** consisting of thin-walled parenchyma; the cells contain polygonal starch grains, embedded in a matrix of protoplasm: in the peripheral yellower portion of the endosperm the starch grains are more closely packed than in the central whiter portion.

iii. The **Embryo,** which is in close apposition to the endosperm: the part which is in contact with it is the **Scutellum** (cotyledon); it extends over the whole surface of contact, and almost completely surrounds the

body of the embryo itself. Note (*a*) the central attachment of the scutellum to the body of the embryo; (*b*) the vascular bundles, which form a connection through it; (*c*) the **Epithelium** of peculiar structure, which faces the endosperm.

The body of the embryo consists of—

a. An **Apical bud,** with several sheathing leaves, which surround the apical cone.

b. A **Radicle,** having similar arrangement of the meristem to that of the older root (*cf. infra.*). Outside the radicle, and continuous with the root-cap, is a root-sheath or **Coleorhiza,** the existence of which shows the endogenous origin of the radicle.

III. Cut sections of the endosperm, and treat with solution of iodine. Note the polygonal starch grains (blue), and the protoplasmic matrix (brown).

Germination.—I. Compare plants which have been germinated for different periods: the following facts in the history of germination may be observed:—

1. The fruit swells.

2. The outer coat ruptures opposite the apex of the radicle, which soon protrudes and bursts through the coleorhiza also, which appears as an irregular ring round the young root.

3. The rupture of the coat extends upwards to the point opposite the apical bud, which also emerges.

4. The root elongates, and forms lateral roots: other lateral roots (usually two) appear above the insertion of the scutellum: these soon equal the primary root in length. Hence there is no well marked tap root.

5. Leaves of the plumule unfold, and gradually turn green.

IV. From a young plant with leaves about three inches long cut longitudinal sections, as above: mount in water, and irrigate with solution of iodine. Observe—

1. That in the neighbourhood of the surface of the scutellum the starch grains are in course of demolition, and that the central part of each is first attacked.

2. That no starch grains are to be seen in the epithelium of the scutellum.

STEM. *HERBACEOUS TYPE.*

I. Cut transverse sections of an internode of a well-grown stem of *Zea Mais;* mount in water.

N.B.—Fresh material may be used, but stems preserved in alcohol are preferable. When fresh, the tissues are crowded with air bubbles. The sections should be cut from the upper part of one of the lower internodes, otherwise the vascular bundles may be found to be imperfectly developed.

Examine with a low power, and, beginning the study of the tissues at the periphery of the section, observe—

a. A single layer of **Epidermis,** having the usual characters: immediately below this are—

b. Irregular groups of **Sclerenchyma** with thick lignified walls: internally lies—

c. A mass of **Parenchyma,** which forms the groundwork of the whole section: embedded in this are—

d. Numerous **Vascular bundles :** note that they are smaller, but more numerous near the periphery than at the centre; also that the position of the parts of the bundles relatively to the centre of the section is uniform.

Treat a section with Schulze's solution: put on a high power, and examine in detail the several tissues above-named.

a. The **Epidermis** appears as a definite layer of cells of unequal size, without intercellular spaces. Note a well-marked **Cuticle** (brown). Here and there may be found **Stomata**, with two small **guard-cells** and two **subsidiary cells** (the structure and development of the stomata will be studied in the leaf; p. 116).

b. The **Sclerenchyma** consists of cells with thick, highly refractive walls, which stain yellowish brown with Schulze's solution (lignified). Note that it does not occur immediately below the stomata, but, as usual, there is there an intercellular space (respiratory cavity).

c. The **Parenchyma** consists of cells with thin cellulose walls (blue with Schulze's solution). At the angles where the cell-walls meet are **intercellular spaces**. The external layers have abundant protoplasm with **chlorophyll-grains.** These are less frequent in the inner layers, while in the central parenchyma the protoplasm is hardly appreciable.

d. For the minute study of the **Vascular bundles** select one of the largest central bundles. The section must be thin. The most prominent elements in the bundle are—

i. Four large **Vessels** of the **Xylem,** arranged like a V, with the angle towards the centre of the stem: of these the two smaller are developed first. Compare sections of young stems.

In many Monocotyledons the arrangement of the constituents

of the xylem in the form of a V is much more plain than here, *e.g.*, *Asparagus*. In other cases (*e.g. Calamus*) this arrangement is not to be seen.

The vessel nearest the centre of the stem has **annular** thickening: in old stems it is partially surrounded by an intercellular space, while the rings often become detached, in which case the vessel is not easily seen in transverse sections. Next this is a **spiral vessel:** the remaining two have thinner walls with **pitted** marking, and large cavity.

Surrounding the pitted vessels, and between them, are—

ii. A number of **Tracheides** with pitted lignified walls, and no cell-contents. Surrounding the intercellular space above described is—

iii. A group of parenchymatous cells with thin cellulose walls. These may be regarded as **Xylem Parenchyma.**

The **Phloem** portion of the bundle lies between the limbs of the V-shaped xylem, and is easily recognised by the thin cellulose walls characteristic of **Soft bast.** It consists of—

iv. Elements with large cavities, in which transverse septa (sieve-plates) often occur. These are **Sieve-tubes.**

v. Smaller cells (**cambiform**) between the sieve-tubes.

Surrounding the above tissues of the xylem and phloem is a **Sheath of sclerenchyma.** Transitional forms may be found on its internal side, between sclerenchyma, and certain of the constituents of the bundle.

II. Cut longitudinal sections of the same, treat as before, and observe—

a. The **Epidermis** composed of oblong cells.

b. The prosenchymatous cells of the **Sclerenchyma.**

c. The **ground parenchyma** with roundish cells.

d. The **Vascular bundles** pursuing a longitudinal course parallel to one another, without lateral fusion.

In the **Xylem** observe—

i. The **annular, spiral,** and **pitted** vessels, and note, especially in the latter, the clearly-marked joints, pointing to their origin from a succession of cells.

ii. The pitted **Tracheides.**

iii. The thin-walled **Parenchyma.**

And in the **Phloem** (which is easily recognised by its cellulose walls, blue with Schulze's solution) distinguish—

iv. The **Sieve-tubes,** which have a wide cavity, intercepted here and there by transverse **sieves.**

N.B. If it be found difficult to distinguish the sieve-plates, a fresh section may be treated with potash; the character of the sieve-plate is then more easily seen.

v. The **Cambiform cells,** which are narrow and parenchymatous.

Note the prosenchymatous constituents of the sheath of **Sclerenchyma,** and observe transitional forms between these and the pitted **Tracheides** (ii.) with square ends, which belong to the xylem.

III. Cut successive, thick transverse sections through a **node:** treat them with strong potash [or better, soak them for twenty-four hours or more in dilute potash]; mount in glycerine, and examine with a low power.

Observe that the vascular bundles here form a dense plexus, in which may be recognised—

1. Branching, and anastomosis of the bundles of the main axis with one another, at the base of the internode.

2. Entry of the bundle-system of the leaf-trace, and of its axillary bud, into the main axis, in which the bundles at first pursue an irregular horizontal course.

3. Anastomosis of these bundles with those of the main axis.

The result is a thorough **intercommunication** of the several systems of bundles, one with another, **at the node.** This modification of the type of bundle arrangement characteristic of the Monocotyledons is the rule in those of the group which have long internodes.

Observe that the structure of the individual bundles at the node differs from that in the internode, the change depending upon—

1. The sheath of sclerenchyma being relatively larger.

2. The irregularities of vascular arrangement resulting from the fusion of bundles.

IV. Cut longitudinal sections through a node in planes parallel to the median plane of the leaf and axillary bud: treat as above, and observe—

1. The branching and fusion of the longitudinal bundles of the internode at the node.

2. The entry, horizontal course, and fusions of the bundle system of leaf, and axillary bud.

Note that the plexus of bundles at the node does not extend far in a perpendicular direction.

V. Apex of stem, to show the fundamental arrangement of the vascular system. Cut median longitudinal sections through the apex of a young plant of Maize,

or of a foliage branch of an old plant: treat with strong potash [or better, with dilute potash for twenty-four hours]: examine with a low power, and observe, if the section be median—

1. The **Apical cone** (*punctum vegetationis*).

2. **Leaves,** in successive stages of development, seated laterally.

3. In the older leaves, **Vascular bundles,** which enter the stem.

On following the course of these vascular bundles it will be seen that on entering the stem they proceed at first towards the centre: before reaching it they curve downwards, and finally turning again outwards they approach the periphery of the stem. We thus see that in young stems of Maize the course of the bundles corresponds to the Palm-type, though as the stem grows older, and the internodes develop, the correspondence is less obvious, by reason (1) of the almost straight course pursued by the bundles in the internode, and (2) the complications which arise at the node.

Stem. *ARBOREOUS TYPE.*

I. Examine preparations of the old stem of *Yucca* or *Dracæna*, in which the thin-walled parenchyma has been allowed to rot away, while the vascular bundles remain. On comparing transverse and longitudinal sections of such stems, it may be seen, with the naked eye—

1. That the central **Primary bundles** are isolated, and that the course of each bundle may be traced as starting from below at the periphery of the stem, then

curving towards the centre as it ascends, and finally turning outwards, and passing into a leaf. These are therefore **common bundles.**

2. That the peripheral mass of secondary bundles increases in thickness towards the base of the stem, and has no direct connection with the leaves. These bundles are therefore **cauline.**

II. Cut transverse sections of the stem of *Dracæna* at a point one foot or more from the apex, and mount in glycerine. Examine with a low power, and observe—

1. A well-marked **Epidermis.** Beneath this—

2. A band of **Cork** (*cf.* Elm).

3. A broad belt of **Cortical parenchyma,** many cells of which contain crystals (Raphides &c.) Here and there a vascular bundle will be seen in the cortex, these are bundles of the leaf-trace, passing inwards from the leaves.

4. At the inner limit of this is an actively dividing **Meristematic ring,** which gives rise internally to new vascular bundles, and externally to fresh cortical cells. The new bundles thus formed are **cauline** (*i.e.* have no direct connection with the leaves), and are embedded in lignified ground tissue. These together form a dense ring.

5. Centrally, an arrangement of thin-walled **Parenchyma** and **Vascular bundles,** similar to that in the internode of Maize.

Note the passage of these central bundles outwards to the bases of the leaves. They are **common** bundles. Note also the mode of formation of the cauline bundles (*cf. Hippuris*).

Transverse sections should also be cut immediately below the apical tuft of leaves. Here the secondary thickening will not have begun, the arrangement of tissues resembling, in all essential points, that in the internode of the Maize.

LEAF.

Note the phyllotaxis in the Maize; the leaf is sessile, and sheathing in its lower half, with a ligule at the apex of the sheath; lamina, form lanceolate, margin entire, ciliate, midrib well marked; venation parallel; upper surface hirsute; lower glabrous.

I. Cut transverse sections of the lamina; mount in water, or dilute glycerine.

Other sections may be treated with alcohol to expel the air bubbles (the chlorophyll will, at the same time, be dissolved out), and be mounted in Schulze's solution, and kept for comparison with the above.

Examine with a low power.

The section presents a sinuous outline, corresponding to a certain extent to the arrangement of the main vascular bundles. At the mid-rib the section widens out. Note the following arrangement of tissues:—

1. Covering both surfaces of the leaf is an **Epidermis,** resembling that of the stem, but bearing **hairs** of various form, mostly simple, conical. The largest of them are surrounded at the base by an outgrowth of the neighbouring epidermal cells.

Note the **Stomata** on both surfaces, with small guard cells, surrounded by two subsidiary cells (*cf. infra*).

2. **Vascular Bundles** of various size, which, in the

thinner part of the lamina, lie in a median position between the two epidermal layers. The largest of these correspond in structure to those of the internode, the smaller ones are reduced forms of the same type. Note that the spiral and annular vessels (*i.e.* protoxylem) are nearer the upper surface of the leaf.

Between the epidermis on either side, and the larger bundles, are masses of **Sclerenchyma,** which, together with the bundles, form complete bridges of rigid tissue between the two epidermal layers.

3. The spaces between the tissues, hitherto considered, are filled with **parenchyma** (**Mesophyll**), which may either be (*a*), green (containing chlorophyll); or (*b*), colourless (without chlorophyll).

a. The green chlorophyll-containing parenchyma fills up the greater part of the space; intercellular spaces occur in it.

b. The colourless parenchyma occurs (i.), as a sheath, without intercellular spaces, surrounding each bundle (bundle-sheath); (ii.) as groups of cells immediately below the epidermis; these are more common towards the central part of the leaf. At the mid-rib this tissue forms the bulk of the structure.

II. Cut transverse sections of the leaf-sheath, and treat as the above. Compare the arrangement of tissues with that of the lamina, and of the stem. Note that colourless parenchyma preponderates.

III. Treat a piece of the thin peripheral part of a leaf (which has been previously bleached in alcohol) with potash till it is transparent; mount in glycerine, and examine under a low power. Observe—

1. The **parallel** course of the **Bundles.**

2. Their frequent **lateral fusion,** by means of small branch bundles.

3. The absence of **Stomata** above the vascular bundles, and their arrangement in rows in the spaces between them.

4. The various forms of **Hair;** and especially the conical unicellular hairs, which give the ciliate character to the margin of the leaf.

IV. Cut thin tangential sections from the under surface of the lamina, so as to remove, if possible, only the epidermis. Treat with potash, and mount in glycerine. Observe—

1. The ordinary cells of the **Epidermis** of oblong form, and with sinuous outline.

2. Short cells between the ends of these, which often project perpendicularly to the surface as **Hairs** of various form.

3. The **Stomata** holding the same position as (2) relatively to the oblong epidermal cells.

Observe with a high power the structure of the stomata. They consist of—

a. Two narrow **guard-cells,** which inclose the **pore.**

b. Two triangular **subsidiary cells,** which completely surround the convex side of the guard-cells.

Compare this view of the stoma with the same structure as seen in transverse sections of the lamina.

V. Cut tangential sections of the upper surface of the lamina. (1). Mount some, and examine them under a low power. (2). Treat others with nitric acid; dry them, and ignite on platinum foil over a spirit lamp. Mount the ash in water, and examine under a low power. The structure will resemble that of (1).

Treat with acetic acid—no evolution of gas.

Treat with nitric acid—it is not dissolved.

The residue is a **silica-skeleton** of the epidermal tissues.

VI. *Development of Stomata.*—Take a young leaf from a bulb of *Hyacinthus orientalis* in which the leaves have not yet protruded more than about one inch from the apex of the bulb. Strip off pieces of the epidermis (or cut tangential sections at successive points) starting from the apex, and proceeding to the very base. Mount in glycerine, and examine under a high power.

i. Starting at the basal part, cell-division will be found to be proceeding actively in the epidermal tissue; the walls are thin, and protoplasm copious. The epidermis consists of—

a. Larger oblong cells.

b. Short, nearly square cells.

The cells are arranged in regular longitudinal rows.

ii. At a short distance from the base, the difference in size of (*a*) and (*b*) increases; some of the square cells may be seen to be divided by a thin longitudinal wall, into two equal halves (guard-cells of the stoma).

iii. Further up again, this division wall may be seen to be thicker at its central part, while the whole outline of the pair of guard-cells tends to become circular.

iv. Again further up, the division wall will be seen to have split, so that a channel is formed between the guard-cells into the internal tissues of the leaf. This channel is the **pore** of the stoma.

v. Near the apex of the leaf the mature stomata may be seen of circular outline; their guard-cells are sausage-

shaped, and surround the nearly circular pore. The cells of the epidermis remain oblong as before.

It will be seen that the stoma of *Hyacinthus* is of simpler structure than that of the Maize. It is more difficult to trace the development of the latter; but it may be done in the same way in a foliage bud. The main point of difference is that after the mother-cell of the stoma has divided to form the two guard-cells, two other cells are cut off from the neighbouring epidermal cells (subsidiary cells). These lie parallel to the guard-cells.

Further, the epidermis of the Maize is complicated by short cells, which appear in irregular groups among the ordinary epidermal cells. This is a common character among the Grasses.

ROOT.

I. Cut transverse sections of the root of *Hyacinthus orientalis.* (N.B. An old root must be taken, and the sections should be cut as far as possible from the apex). Treat them with potash, and mount in glycerine. Starting from the outside, note successively—

1. An **Epidermis,** not well marked. Note here and there cells, which have grown out perpendicular to the surface as **root-hairs.**

2. A thick band of **Cortical parenchyma,** consisting of rounded cells with intercellular spaces; in old roots the outer layers of this tissue become disorganised and distorted. The inmost layer of this tissue differs in structure from the rest, and is called—

3. The **Bundle-sheath:** the radial walls of this layer present the characteristic appearance of a black dot, and are cuticularised. Within this is—

4. A layer of thin-walled cells (the **Phloem-sheath** or **pericambium**), which immediately surrounds—

5. The central **Vascular cylinder.** This consists of groups of tissue of two sorts.

A. **Xylem-tissues,** easily recognised by their dark lignified walls. They are arranged in a series of groups of indefinite number, which abut externally on the pericambium, and extend inwards, till they meet internally, and form a central mass. The chief constituents are vessels of various form.

As may be seen in transverse sections of young roots, the smaller peripheral members of each group are formed first (**protoxylem**), and have spiral thickening; then successively the larger vessels towards the centre. Between the peripheral groups of the xylem, and alternating regularly with them may be seen—

B. The **Phloem-tissues,** which are groups of elements with small cavity, and bright cellulose walls.

II.—Cut radial longitudinal sections of the same root: treat in the same way, and observe the several tissues above described. The whole root will be seen to be composed of similar elements to those found in the stem.

Transverse sections should also be made of the root of the Maize. The main features of the section are the same, though the structure differs in several minor points from that of the root of Hyacinth. Thus, in the Maize root there is a parenchymatous pith, and the xylem abuts directly on the bundle-sheath.

In these sections may be found the point of junction of lateral roots with the main root. It may be seen that the former originate from the pericambium of the main root, and that they break through the bundle-sheath, cortical tissue, and epidermis; also that their vascular tissue is continuous with that of the main root; the activity which produces them begins opposite a phloem-mass of the main root, and not opposite a xylem-mass,

as is usually the case (*cf.* Dicotyledons). This is to be connected with the fact that the xylem-groups in the Maize (and in most Grasses) abut directly on the bundle-sheath.

Apex (*punctum vegetationis*).

It is not easy to cut longitudinal sections of the apex of an ordinary fully developed root without embedding. The arrangement of the meristematic tissues is, however, the same in young as in old roots; it is therefore more convenient, and quite as successful, to cut longitudinal sections of the apex of the young lateral roots, which are to be found growing horizontally out of the nodes of the Maize plant. Or, if fitting material for this be not at hand, longitudinal sections may be made of the radicle of the embryo, in seeds which have been previously soaked for several hours in water.

Adopting one of the above methods, cut longitudinal median sections of the apex of the root. Treat them for ten minutes with dilute potash: neutralise with acetic acid, and mount in glycerine.

N.B.—The section must be accurately longitudinal and median, *i.e.* the section must include the organic axis of the root, around which the several tissues are symmetrically arranged.

In a median section the following arrangement of tissues will be visible.

1. A central mass of tissue, clearly defined laterally, and rounded off at its apex, which is at some distance below the external apex of the root: this is the **Plerome** cylinder. If this tissue be traced back into the older part of the root it will be found that its central part is continuous with the parenchymatous

pith, while its peripheral part develops into the vascular ring. Note rows of larger cells, which may be traced back as continuous with vessels of the xylem. In the central portion of the plerome are intercellular spaces, which appear black in sections from fresh material, being filled with air.

2. Surrounding the plerome is a broad band of tissue with intercellular spaces, which appear as above. This is the **Periblem,** which is the formative tissue of the cortex.

3. Outside this is a single layer of cells somewhat elongated radially, and with a thick outer wall: this is the **Dermatogen** or formative tissue of the epidermis.

If the section be accurately median it will be possible to trace (2) and (3) upwards, till, immediately above the apex of the plerome, they merge into a single layer of cells: thus the formative tissue from which the epidermis and cortex are derived, is represented at the apex by a single layer of cells.

4. Outside the dermatogen, at the apex of the root will be found another formative tissue, the cells of which divide parallel to the surface of the dermatogen: this is the **Calyptrogen** layer, which is formative of the tissues of the **Root-cap.** The latter appears as a mass of parenchyma, covering the whole apex of the root: the outer cells of it will be seen to be undergoing disorganisation, and mucilaginous degeneration of the cell-walls.

REPRODUCTIVE ORGANS.

Development of the Flower.

I. Examine young **Capitula** of the Sunflower with the naked eye: they occur in the same positions as the vegetative apical bud, but differ externally from these—

1. In their greater bulk, and more especially in their diameter being larger than in these.

2. In their colour, which is usually darker.

3. In being covered externally by a large number of imbricated **Bracts** (or **hypsophyllary leaves**), which together form the general involucre.

Select a very young capitulum, that is, one in which these characters can be recognised, but are not as yet very pronounced, and, having removed the largest external bracts, cut from it median longitudinal sections: treat with potash for about ten minutes, and mount in glycerine: observe with a low power—

1. That in outline and general arrangement of parts the sections resemble those of the vegetative bud, but that the apical cone is broader, and more flat.

2. That the surface of the cone has an irregular outline, owing to the formation of a series of appendicular organs, which are developed in **acropetal order,** *i.e.* the smallest or youngest are nearest the apex, while on passing towards the periphery the size regularly increases.

Put on a higher power, and study these organs in detail.

Beginning at the centre: if the capitulum be young enough, there will be found, as in the vegetative bud, a naked **apical cone,** with a similar arrangement of tissues to that there observed. Passing from the centre, the external surface assumes an undulating appearance owing to the formation of—

1. **Bracteoles,** leaf-structures, which arise similarly to the leaves as above observed, by outgrowth of the epidermis and subjacent tissue: as they grow older they curve towards the centre. [Note the formation of **hairs** of various types from single cells of the epidermis: this being a good opportunity for tracing their origin.]

2. The rudiments of **Flowers,** which appear in the axils of the bracteoles [*i.e.* on the side nearer the apex]. These are likewise produced from the epidermis and subjacent tissue, they are morphologically **axillary branches.**

The development of the latter into the complete flower must be carefully studied, by comparison of those nearer the centre with older flowers nearer the periphery of the capitulum, or on capitula of various ages. It is obvious that flowers which have been cut in median section will be best fitted for this study. Note the following successive stages of development—

(*a*). Form of papilla, conical.

(*b*). Apex becomes flattened.

(*c*). Periphery of the flattened apex rises into a whorl of five small lobes; these are the **Petals,** which are in the mature flower united in a gamopetalous **Corolla.**

(*d*). Between the corolla, and the now depressed

apex, rises a fresh series or whorl of five lobes, these are the young **Stamens.**

About this stage may be seen externally, below the corolla, a slight protuberance on each side of the flower (as seen in section). This is the first appearance of the **Calyx,** which consists in the mature flower of two scaly sepals.

N.B.—This order of appearance of the floral whorls is not normal, but is the rule in the order *Compositæ.* In the large majority of plants the calyx is developed first, then the corolla, and then the stamens.

(*e*). Within the whorl of stamens there arise, at the margin of the now much depressed apex, the last series of floral organs, viz., two **Carpels,** which arch over the apical depression, and thus close in the cavity of the **inferior ovary.**

(*f*). All the organs increase in size, while from the base of the cavity of the ovary, a papilla arises, which develops into a single **anatropous Ovule,** with one **Integument,** and small **Nucellus.**

(For the development of the ovule *cf. Helleborus,* p. 130.)

Cut horizontal (*i.e.* transverse) sections of a capitulum: treat as before: examine with a low power.

Note the arrangement of bracteoles, with young flowers in their axils, round the central naked apex. The youngest flowers will appear simply circular in outline (simple papillæ of stages *a* and *b*): older flowers will show successively—

(i). The five papillæ of the **Corolla** (petals) uniting at an early stage into a gamopetalous corolla-tube. (Stage *c.*)

(ii). Five **Stamens,** alternating with the petals. (Stage *d.*)

(iii). Centrally **two Carpels.** (Stage *e.*)

II. Take a mature flower of *Helleborus fœtidus.* Observe, and remove successively—

1. The five **Sepals,** polysepalous, regular, inferior, and herbaceous.

2. **Petals,** number various, polypetalous, tubular, inferior.

3. **Stamens,** numerous, hypogynous, free.

4. **Carpels,** number various, apocarpous, superior.

Examine a single **Stamen,** and observe that it consists of—

a. A thin stalk—the **Filament.**

b. A two-lobed head—the **Anther.**

In a fully open flower note the lateral, longitudinal dehiscence of the anthers, and the dusty **Pollen** thus liberated.

Examine a single **Carpel;** it consists of a lower thicker portion, terminated by a thin curved portion (the **Style**); on the inner surface at the top of the style is the **Stigma.**

Slit a carpel open along the dorsal side, turn back the flaps, and observe the numerous **ovules,** attached, in two rows, to the ventral side of the carpel.

Stamen.

III. All the following preparations should be made from materials hardened in alcohol, or better, fixed with saturated solution of picric acid, and then washed and hardened with alcohol.

A. Cut transverse sections of a flower bud of *Helle-*

borus fœtidus, which was just ready to open, taking care that the anthers shall be cut through transversely. Neglecting the other parts, mount the sections of the **anthers** in glycerine, and examine with a low power. Note—

1. The general outline of the section, and compare it with the form of the bi-lobed anther, as above observed.

2. The two large cavities one in each lobe.

3. The **partial Septa,** which originally divided each cavity into two **Pollen-sacs** or **Microsporangia;** the anther has thus orginally four pollen-sacs, and these may sometimes be found still distinct even in almost mature anthers (*cf.* development of anther).

4. A single small **Vascular bundle** lying symmetrically between the cavities, in the central part (or **Connective**) of the anther.

5. **Pollen-grains** or **Microspores,** mostly to be found lying free in the glycerine.

Put on a high power, and observe that—

1. The wall of the anther is composed of—

(*a*) An external **Epidermis** with a well-marked **cuticle.** Within this—

(*b*) A layer of cells with a **fibrous thickening** of the walls.

(*c*) Immediately within (*b*) a narrow ill-defined band, consisting of the remnants of a transitory layer of cells, the **Tapetum.**

2. A point in the wall of each cavity, opposite the partial septum, where the cells are smaller, and the inner layer not spirally thickened; this is the **point of dehiscence** of the anther.

3. **Pollen-grains** or **Microspores** are almost spherical, with smooth walls, and granular protoplasmic contents, in which may be made out, with difficulty, **two nuclei.**

B. Mount in half glycerine half alcohol some almost mature pollen of *Fritillaria imperialis,* which has been previously preserved in alcohol, and examine with a high power. The grains have a smooth wall, and in the granular protoplasm may usually be seen **two nuclei.** N.B.—If the grains be stained with hæmatoxylin before mounting in glycerine and alcohol, the nuclei will be more easily made out.

Mount and examine, as types of the various forms of the grains, the pollen of *Helianthus, Althæa, Cucurbita, Œnothera, Orchis* (pollen-masses or pollinia), *Mimosa, Cichorium,* &c.

C. In order to observe the germination of the pollen-grains, and formation of the pollen-tubes, use may be made of the moist chamber, described on p. 16.

Mount some pollen-grains of *Helianthus* in one hanging drop of a weak solution of cane-sugar in water (about 5 per cent.). Examine them with a high power, and note their form and the external configuration of their walls.

Keep them at an ordinary temperature in the dark for a few hours: on again examining them, many will be found to have put out **Pollen-tubes,** filled with granular protoplasm, in which one or more **nuclei** might be detected.

The same method may be used for the pollen of other plants, *e.g.* Orchids, species of *Tulipa, Fritillaria, Nymphæa,* &c. It

will be found that the time of appearance of the pollen-tube will vary in different cases ; also that to obtain good results solutions of sugar of different strengths will have to be used. In most cases a solution of 10 per cent. or less will be found suitable.

Development of Anther and Pollen.

If transverse sections be made from very young buds, and successively from older ones up to the mature flower, the development of the anther and of the pollen may be traced.

The material should be preserved in absolute alcohol (or strong methylated spirit), and the sections should be treated with half glycerine, half alcohol ; this should be left exposed to the air in a watch-glass, so that the alcohol may evaporate ; mount in pure glycerine.

(Anhydrous staining reagents may be employed.)

By following this method, sections may be prepared illustrating :—

1. The formation of the four masses of tissue in the anther (two in each lobe), each of which subsequently becomes differentiated into :—

(*a*) A peripheral coat of cells of the **tapetum,** which take no direct part in the formation of the pollen, and—

(*b*) A central mass of **pollen-mother-cells.**

2. The division of each of the pollen-mother-cells into four **special-mother-cells,** by the gradual ingrowth of the wall of the mother-cell.

3. The separation of the members of the tetrads thus formed, and their subsequent development as **pollen-grains.**

4. The gradual disorganisation of the **tapetum.**

5. The development of the wall of the anther, as above described, and breaking down of the septum between the pairs of pollen-sacs.

Compare similar preparations of the young anthers of *Tradescantia,* and note the division of the pollen-mother-cells, without any gradual ingrowth of the wall.

Observe, as far as possible, the divisions of the nuclei of the pollen-mother-cells first into two, then into four ; also the two nuclei in the mature pollen-grain.

Carpel and Ovules.

IV. The following preparations must be made from materials hardened in absolute alcohol (or methylated spirit):—

Strip off the sepals, petals and stamens from an open bud of *Helleborus fœtidus*, and cut transverse sections of the **Carpels.** Treat the sections with one half pure glycerine, one half alcohol, and let the alcohol evaporate gradually. Mount in pure glycerine.

Strasburger recommends that the transfer to pure glycerine should be made before the sections are cut.

Examine first with a low power, and observe—

1. The **Carpel,** having a structure not unlike that of an ordinary leaf. Note the **suture** or junction of the two margins of the carpel which thus incloses a central cavity.

2. The **Ovules** (**Macrosporangia**) seated in this cavity, and attached near the margins of the carpel (it has already been noted that there are two rows of ovules in each carpel, therefore at most only **two** ovules appear in each section).

The form of the ovule is **anatropous;** it consists of the following parts:—

(*a*) The **Funiculus**, or stalk, which adheres through the greater part of its course (as the **Raphe**) to the body of the inverted ovule. A procambium bundle, connected with a bundle at the margin of the carpel, traverses it longitudinally.

The body of the inverted ovule consists of—

(*b*) One **Integument** several layers of cells thick, united with the funiculus, and covering the body of the ovule completely, excepting a narrow channel (**Micropyle**) near the apex of the ovule. Within this lies—

(*c*) The **Nucellus,** a mass of cellular tissue in which is embedded—

(*d*) The **Embryo-sac** (**Macrospore**), a large oval cell, situated a short distance below the apex of the nucellus.

Examine the embryo-sac with a high power, and observe—

1. The granular, vacuolated protoplasm which fills it; embedded in this are to be found—

2. A large central **nucleus.**

3. At the micropylar end of the embryo-sac, **three cells,** with clearly defined nuclei. Two of these (the **Synergidæ**) fill the apex of the sac, the third (the **Oosphere**) being placed laterally, a little below the apex.

4. At the posterior end of the sac are three cells (the **Antipodal cells**), also with clearly defined nuclei.

Note the **Tapetum,** consisting of cells more or less disorganised, which partially or completely surround the embryo-sac.

If similar sections be cut from buds of *Helleborus fœtidus* of various ages, and be treated in the same way, the development of the ovule, and more especially of the embryo-sac, may be followed, and the various stages of it may be observed.

Make similar sections of the ovary of species of *Lilium*, or *Yucca*, and compare them with the above. With the exception of a second integument being present in these cases, the structure of the ovule will appear to correspond to that of *Helleborus.*

Fertilisation.

I. Cut median vertical sections through the stigma and upper part of the style of a flower of *Datura Stramonium* which has just faded. Mount in dilute glycerine, and examine first with a low power. Note—

1. The closely-packed tissue covering the **Stigmatic surface,** the superficial cells of which are slightly elongated perpendicularly to the surface as hairs.

2. The more lax **Cortical tissue** of the style, with numerous intercellular spaces, which appear dark under the microscope.

3. A central band of more transparent tissue without intercellular spaces (**Conducting tissue**).

4. Small vascular bundles, two in number, running up the style; these may or may not be present in the section, according as it has been cut.

5. **Pollen-grains** adhering to the surface of the stigma; from them **pollen-tubes,** similar to those grown in sugar solutions (*cf.* p. 127) may often be traced penetrating the tissue of the stigma.

Now gently boil the sections in the dilute glycerine over a spirit lamp, and examine again. Observe—

1. The **Pollen-grains** as before.

2. **Pollen-tubes,** which may be traced from them through the now more transparent tissues of the style; they may be recognised by their densely granular contents.

Other flowers besides the above may be used *e.g.* species of *Œnothera*, &c., or any flower in which the style and stigma are of considerable size.

II. Pick out gently a number of ovules from an ovary of a flower of *Datura Stramonium*, which has just faded, and mount in dilute glycerine. Observe—

1. The **Campylotropous Ovules,** with curved body.

2. **Pollen-tubes,** which are often to be found with the end applied closely to the micropyle.

Strasburger observed the process of fertilisation itself directly in *Torenia asiatica*, *Gloxinia*, and also in Orchids, *Monotropa*, and *Pyrola*. His method was to open the ovary of a flower a short time after pollination, and detach and mount the ovules in a 3 per cent. solution of sugar.

Development of the Embryo.

i. *Dicotyledon.*

Pick out the ovules from an ovary of *Capsella Bursa-pastoris*, which has attained about half the ultimate size of the mature fruit. Treat with dilute potash, and examine with a low power. Observe—

1. The form of the ovule (**campylotropous,** *i.e.* with a curvature of the body of the ovule).

2. The **Funiculus,** or stalk.

3. The **Integuments.**

4. The **Micropyle,** not very easily seen: a **pollen-tube** may often be observed entering the micropyle.

5. A large central cavity (the **Embryo-sac**), which is curved like the whole ovule. In this may be seen, more or less distinctly—

6. The **Embryo.**

To study the structure of the embryo, either longitudinal sections of the ovule must be cut, and the embryo be thus laid bare, or the embryo must be removed from the ovule. The former is the more accurate method, though the latter is much the easier: we will therefore adopt the latter.

Press gently with a needle upon the cover slip of the above preparation, so as to burst the ovules: the embryo will escape in some cases without injury; neutralise the potash with dilute acetic acid. The structure of the embryos, which now lie freely suspended in the fluid, may be easily studied.

Apply the same method for the preparation of embryos, from ovaries of various ages, both younger and older than that first taken. A series of preparations may thus be obtained illustrating various stages of development of the embryo, such as are figured in ordinary Text-books.

Note more especially the following successive stages of development:—

1. The **Suspensor,** consisting of one or more cells, and terminated by a single **Embryonic cell.**

2. The embryonic cell divided into **octants** arranged in two tiers, the terminal cell of the suspensor (**Hypophysis**) encroaching between the four lower octants.

3. The octants so divided up as to form three layers of cells, which have been distinguished as (*a*) the external **Dermatogen;** (*b*) the **Periblem;** (*c*) the central **Plerome.**

4. The two **Cotyledons** formed by lateral outgrowth from the upper tier of octants, the apex of the **Radicle**

derived from the hypophysis, the hypocotyledonary stem from the lower tier of octants.

5. Other parts as before. The **Apical cone** or **Plumule** formed between the cotyledons.

ii. *Monocotyledon.*

Treat ovules of *Alisma Plantago* in the same way, and observe the following stages of development :—

1. **Suspensor** and **Embryo** consist of a single short series of cells, produced by transverse divisions.

2. The terminal cell divides longitudinally into four (first tier).

3. The second, third, and fourth cells from the end also divide successively (second, third, and fourth tier).

4. The cells of the body of the embryo divided (as in *Capsella*) so as to form three layers—(*a*) external **Dermatogen,** (*b*) **Periblem,** (*c*) central **Plerome.**

5. A lateral depression of the surface, at the level of the second tier. At the basal lip of this the **Apical cone** of the plumule is formed.

The single **Cotyledon** is formed from the first tier.

The **Radicle** from the third tier.

The **Apex** of the root from the fourth tier.

Compare these results with those obtained in *Capsella.*

For obtaining preparations of the embryo *in situ,* and of the **Endosperm** surrounding it, the ovary of species of *Potamogeton* will be found to be good material: it should be previously hardened in spirit.

Cut longitudinal sections of a single carpel, parallel to the flattened sides: they should not be cut too thin. Mount in glycerine, and examine with a low power.

One of the sections will probably be found to include—

1. The **Embryo-sac,** in which are contained—

2. The **Embryo,** with a very short suspensor, the basal cell of which is greatly enlarged.

3. The **Endosperm,** a tissue which lines the embryo-sac, and varies in appearance according to the stage of development of the ovary.

II. GYMNOSPERMS.

VEGETATIVE ORGANS.

EXTERNAL CHARACTERS.

Take a branch of *Pinus Sylvestris*, cut in autumn, and which includes at least four years' growth.

N.B.—The limits of each year's growth may be recognised externally as those points where (false) whorls of strong lateral axes are developed ; and the portion of stem lying between two such whorls may be considered roughly as representing one year's growth.

I. Consider first the growth of the year in which the branch was cut, *i.e.* the part above the youngest whorl of lateral axes. At its apex is a large **Bud,** surrounded by a variable number of smaller **Lateral buds.**

From a bud, which has been treated with alcohol to remove the resin which covers it, detach some of the brown **Scale-leaves,** which cover it externally. Note—

1. The succulent base of these scales.
2. Buds in their axils.

Compare these winter buds with some of the same which have been cut in late spring. The brown scale-leaves will be found to have fallen off, leaving their succulent bases still persistent ; in

the axils of these will be seen the axillary buds above noted. The main axis of the bud has become elongated by extension of the tissues.

In studying the growth of the current year, bear in mind that it has been derived from a bud, which had a similar structure to that which is now seated at its apex. Examine the stem of the current year externally, and note—

1. The thick **Main axis,** more or less succulent in appearance; its surface is marked by longitudinal grooves.

2. The brown tooth-like bases of the scale-leaves of the bud, best seen at the lower part of the internode.

3. In the axils of these, especially at the upper part of the internodes, are **Axillary buds** of two kinds.

a. **Buds with limited growth,** each bearing two acicular **foliage leaves,** surrounded at the base with numerous scale-leaves. These limited foliage shoots occur in the axils of the scales throughout the greater part of the current year's growth.

b. **Buds with unlimited growth,** which are seated close to the apex of the shoot of the current year. They are few in number; their structure has already been observed; each may develop into an unlimited axis.

It may here be observed that both *a* and *b* have a similar origin, both being axillary buds in the axils of the leaves of the main axis of the current year. The apparent difference depends upon the fact that the buds *b* are more strongly developed than *a*.

There is great variety in the character of the leaves in the *Coniferæ*. In some cases only foliage leaves are developed (*Araucaria*, *Juniperus*, *Thuja*). In one case only scale leaves are formed (*Phyllocladus*), while here in *Pinus* we have both scale- and foliage-leaves, the former alone being borne on the stronger axes with unlimited growth, while the latter appear only on the foliage shoots with limited growth. Specimens of different members of the group should be examined and compared.

II. Passing to the increment of growth of former years, *i.e.* to the lower and older parts of the branch, in the external appearance and arrangement of parts they resemble that of the current year. The main axis increases in thickness, and is more obviously ligneous, while the limited foliage shoots drop off, leaving scars which mark their former position.

HISTOLOGY.—THE STEM.

It is best to work with material which has been treated for some time with spirit; by this means the resin, which would otherwise clog the razor, is removed.

I. Cut transverse sections of the **Axis of a bud,** and treat with dilute potash for a few minutes: mount in glycerine.

Meanwhile other sections may be mounted in Schulze's solution: examine with medium or low power, and observe at the centre of the section—

1. The **Pith,** composed of cells, with intercellular spaces, and thick cellulose walls (blue with Schulze's solution); surrounding this a series of groups of smaller constituents: these are—

2. The primary **Vascular bundles.** Note that they are—

a. Separated from one another laterally by bands of parenchyma.

b. Their form is approximately wedge-shaped.

c. That the tissues of which they are composed may be distinguished as—

i. A **Xylem** portion, nearer the centre of the stem, the components of which have thick, dark-looking, lignified walls (yellow with Schulze's solution). These first formed xylem elements, since they differ from those formed later, are distinguished as **Protoxylem.**

ii. A **Phloem** portion, nearer the periphery, with bright-looking cellulose walls (blue with Schulze's solution).

The more minute study of these tissues must be deferred for the present. Outside the ring of vascular bundles is—

3. The **Cortical tissue,** a mass of cells similar in structure to the pith. In this occur large intercellular spaces, which are **Resin-passages.** Since the periphery of the section of the axis of the bud is complicated by great irregularity of outline, the study of the outer tissues will be better carried out in the older stem.

II. Cut transverse sections of the stem of the current year. Mount some in glycerine, others in Schulze's solution. The sections have a wavy outline, the indentations corresponding to the grooves above observed externally. Starting from the periphery of the section, note the following tissues :—

1. **Epidermis,** a single layer of cells, following the wavy outline of the section : the walls, especially the outer, much thickened : externally a **Cuticle.**

2. **Cortical tissue,** consisting of cells with rather

thick cellulose walls (blue with Schulze's solution), and protoplasmic contents with **chlorophyll.** Many cells have recently divided (this is necessary to keep pace with the growth in thickness of the vascular cylinder). Large intercellular spaces (**Resin-passages**) occur here and there, and are lined with small-celled epithelium.

It must be remembered that in the present case the resin itself has been dissolved out by alcohol. Sections should, therefore, be made from fresh material in order to see the secretion *in situ*. It appears amorphous and transparent; it is soluble in alcohol, leaving a slight residue. N.B.—All resins are not so easily soluble.

The secretion stains deeply with tincture of alkanet.

Near the periphery of the cortex will be found a layer of **Cork** and a **Cork-cambium** (*cf.* stem of Elm p. 70), derived from cells of the cortex by their division by tangential walls. The cells of the **cork** have no cell-contents; their walls are coloured yellowish brown with Schulze's solution.

Treat a section with strong sulphuric acid. The walls of the cork retain their sharp contour.

At the bases of the indentations of the margin of the section, and immediately below the epidermis note groups of **Sclerenchyma,** having thick lignified walls (yellow with Schulze's solution).

3. The **Vascular system:** here a complete ring (*cf.* the bud): distinguish as before (*a*) the external **Phloem,** (*b*) the internal **Xylem,** (*c*) the misty layer of **Cambium.**

N.B.—The vascular bundles were seen to be separated in the bud by intervening parenchyma. Here the ring

has been completed by the formation of an **Interfascicular cambium** in the parenchyma between the original bundles.

Observe that the internal limit of the vascular ring is sinuous. The convexities mark the position of the primary bundles; at the apex of these will be found the **Protoxylem.**

4. The **Pith,** consisting of parenchyma, having the same characters as in the bud. No resin-passages.

Put on a high power, and examine the **Cambium.** Note—

i. That the cells are arranged with great regularity in **radial rows.**

ii. That their walls are thinner than those of the surrounding tissues, and are composed of cellulose (blue with Schulze's solution).

iii. That the tangential walls are thinner than the radial.

iv. That the cells have copious protoplasm, in which a **Nucleus** may often be recognised.

These facts point to a repeated division of cells by tangential walls.

Draw carefully, and compare several of the radial series of cells of the cambium. They will be found to coincide with Sanio's law of cambial division, which was first concluded from observations on *Pinus sylvestris.*

Observe, here and there, radial rows of which the cells are more elongated in a radial direction than the rest. These may be traced outwards towards the cortex and inwards towards the pith. They are the **Medullary rays.** Some of them may be traced the whole way to the cortex and to the pith (primary medullary rays),

others only part of that distance (secondary medullary rays).

Note that the cells of the medullary rays at the cambium zone are less elongated radially than in the xylem or phloem; the cambium being the formative point of these tissues.

The mature cells of the ray usually have cellulose walls (blue with Schulze's solution), and granular protoplasmic contents with nucleus. In fact the cells of the medullary rays usually retain their cell-nature.

Follow the radial rows of cambium cells outwards, and note the gradual transition to the permanent tissues of the **Secondary phloem,** the constituents of which are also arranged in radial rows, and have cellulose walls (blue with Schulze's solution). The ring of secondary phloem is cut up into rectangular areas by the **Medullary rays,** which are easily recognised as above directed. Observe that the tissues filling these areas are of three sorts.

i. Elements with cellulose walls, and no very distinct contents; they are radially compressed. These are the **Sieve-tubes,** which compose the greater part of the phloem. The walls are differentiated into layers, and have bright globules attached to them (yellow with Schulze's solution).

ii. Here and there the radial rows of sieve-tubes are broken by single large cells of the **Bast-parenchyma,** which resemble in their characters those of the medullary rays.

iii. Towards the periphery of the phloem are elements similar in form to the sieve-tubes, whose cell contents are brown, and contain crystals.

Note on passing to the periphery of the phloem an increasing irregularity of form of the tissues due to distortion, caused by pressure from without by the cortical tissue upon the vascular system, as it increases in bulk by secondary thickening.

Sclerenchymatous elements are absent from the phloem of the stem of *P. sylvestris.* They are, however, found in the phloem of many of the *Coniferæ*, *e.g.*, *Juniperus*, in which the different tissues are arranged with great regularity.

Follow the radial rows of cambium cells inwards, *i.e.* towards the centre of the stem. Note the transition from thin-walled cambium to the thick-walled tissue of the **Xylem.** If the stem was cut in winter the transition will appear sudden, if cut in summer it will appear gradual. The tissue-elements retain the same arrangements in radial rows, as the cells of the cambium.

Observe that the xylem ring is cut by the medullary rays into wedge-shaped areas. The chief tissue-elements filling these areas are the **Tracheides,** which present the following characters :—

i. They have approximately the same shape as the cells of the cambium from which they are derived.

ii. Their walls are thick and lignified (yellow with Schulze's solution), and are differentiated into layers distinguished optically, and by staining.

iii. They have no cell-contents.

iv. On their **radial walls** (and rarely on the tangential walls) are found irregularities of structure called **Bordered pits,** which are best seen in the xylem formed at the early part of the year. Note the pit-membrane,

which is always present, traversing the pit-cavity in all cleanly cut sections; the pits are therefore not perforated.

Observe near the centre, and bordering on the pith, the **Protoxylem** arranged as above observed in the younger stem. No bordered pits occur in the walls of the protoxylem.

Note the occurrence of **resin-passages** in the secondary xylem, lined as before by thin-walled epithelium, which may be regarded as **xylem-parenchyma.**

III. Cut transverse sections of a **three years old stem** so as to include the whole width of the vascular ring. It is not necessary, however, to have a complete transverse section of the whole stem. Mount in glycerine. Comparing this with what has already been observed in the stem of the current year, note the following differences:—

1. The cortical tissue bears evident traces of tangential extension. This is necessary to keep pace with the increase in bulk of the vascular system.

2. The phloem is thicker, and the constituents of the outer part of it are much distorted and displaced.

3. The xylem has increased in thickness more than any other tissue, so that it is now the chief constituent of the stem. It may be distinguished as being composed of three bands (**annual rings**), in each of which the more central tracheides have large cavity and thinner walls (wood developed in spring); passing outwards there may be seen a gradual reduction of the cavity, and increase in thickness of the walls till a certain limit is reached (autumn wood). Outside the latter is a sudden

transition to the spring wood. At this point is the limit of each year's growth.

IV. Cut radial longitudinal sections of a three years' old stem. Mount some in glycerine, others in Schulze's solution.

The sections should be accurately radial and longitudinal, otherwise the difficulty of study of the tissues is greatly increased.

Beginning at the centre of the stem and passing outwards observe successively :—

1. The **Pith,** consisting of two sorts of elements, both of which are of parenchymatous form.

a. Cells with cellulose walls (blue with Schulze's solution) pitted, with protoplasm and nucleus.

b. Elements of similar form with pitted lignified walls, and no cell-contents.

From the arrangement of these it may be concluded that they had a common origin.

2. The **Xylem** consisting of—

a. **Tracheides** with lignified walls, and no cell-contents. Starting from those nearest the pith (*i.e.* from the protoxylem), and passing outwards, the following forms may be observed, and distinguished mainly by the markings due to unequal thickening of the walls.

i. Tracheides with narrow cavity, and more or less regular annular or spiral marking.

ii. Elements wider than these, and with bordered pits scattered between the spirals.

iii. Normal **Tracheides** with bordered pits only. These form by far the greater bulk of the secondary xylem, and must be carefully studied. Their form is **prosenchymatous.** The greater part of the

cell-walls is of uniform thickness. On these portions of the wall observe with the highest power two intersecting systems of **lines of striation.** In single longitudinal rows are found the **Bordered pits;** each of these appears as two concentric rings, of which the smaller is more strongly marked, and corresponds to the opening of the pit into the cell-cavity.

It must be remembered that we are now observing the radial walls in surface view. Compare the bordered pit as seen here with its appearance when seen in transverse section.

Note the annual rings recognised here, as in the transverse sections, by difference in width of cavity, and thickness of walls of the tracheides of the xylem.

b. Here and there the continuity of the mass of tracheides is broken by a longitudinal **resin-passage,** surrounded by parenchymatous cells (**xylem-parenchyma**), which have cellulose walls and retain their cell-contents.

The whole mass of xylem is traversed radially by plates of parenchyma (**Medullary rays**). Note that they extend only a short way longitudinally, but a long way radially; also that they are composed of cells arranged like bricks in a wall, among which may be be distinguished—

a. Cells with cellulose walls, and protoplasmic contents. The walls of the tracheides which abut on these are unusually wide.

b. Elements, with no protoplasm, and with lignified walls marked with bordered pits.

Both tissue-forms may often be found in the same ray, though rays will often be seen consisting of (*b*) alone.

3. The **Cambium-layer** consisting of elongated thin-walled cells, the ends of which are difficult to observe (*cf.* tangential sections). They have copious protoplasm, and an elongated nucleus.

Note that the medullary rays are continuous through the cambium, and observe the differentiation from the uniform cambium of the ray to the forms (*a*) and (*b*).

In the sections through the cambium of a stem cut in summer, the development of the bordered pits on the walls of the tracheides may be studied. The sections should be treated with Schulze's solution for a long time.

4. The **Phloem** tissues, which are best studied in sections, which have been treated for some hours with Schulze's solution, consist of—

a. **Sieve-tubes,** elongated structures with cellulose walls, those which are radial being marked by numerous circular **sieve-plates,** here seen in surface view. These sometimes stain a sherry brown with Schulze's solution. The ends of the tubes are difficult to observe (*cf.* tangential sections). Their protoplasmic contents are transparent and sparing.

b. **Bast-parenchyma,** cells arranged in longitudinal rows, with cellulose walls, and copious protoplasm.

c. Occasional elements (prosenchymatous or parenchymatous) with brown cell-contents, in which **crystals** are embedded. These are found towards the periphery of the phloem.

Medullary rays will be seen with a similar arrangement to that seen in the xylem. Their cells, which resemble those of the phloem parenchyma in character, are all alike.

5. Externally to the phloem is the **cortical paren-**

chyma, which requires no further notice here. Outside this is cork (and sclerenchyma at certain points), and at the periphery of the section—

6. The **Epidermis.**

V. Cut tangential sections of a three or four years' old branch, and bear in mind that as a rule the central part of the sections is the most accurately tangential, *i.e.* that the plane of section is there most accurately perpendicular to the radius of the stem. Mount as before.

A. In sections which pass through the peripheral part of the xylem observe—

i. The **Tracheides** of prosenchymatous form. No bordered pits (or very few) are seen in surface view, but they may be seen in large numbers in the radial walls (here cut longitudinally) presenting a similar appearance to that seen in transverse sections.

ii. **Medullary rays,** which resemble a section of a biconvex lens. Note that each ray extends only a short distance in a longitudinal direction: in some cases rays consist of only a single radial series of cells, of which only one lenticular cell appears in this section. Occasionally a **resin-passage** is included in a ray.

iii. Longitudinal **resin-passages** (*cf.* radial sections).

B. In sections passing through the cambium will be seen—

i. The **Cambium-cells,** resembling the tracheides in form (prosenchymatous); cell-walls thin; protoplasm granular, with elongated nucleus.

ii. **Cambium of medullary rays,** similar in shape to the cells of the rays: thin-walled, with granular protoplasm and nucleus.

If these sections be treated with dilute potash, and mounted in glycerine, their structure may be more easily made out.

C. In sections passing through the phloem will be seen—

i. The **Medullary rays** as before, but their form is more convex: all the tissues between the medullary rays are derived from cambium-cells of the form above observed. These are—

ii. **Sieve-tubes,** which retain the form of the cambium-cells: the cellulose walls seen in surface view are smooth: those cut longitudinally appear of wavy outline (sieves). The structure of the latter is well seen after treatment with Schulze's solution for twenty-four hours. Contents transparent protoplasm.

iii. **Bast-parenchyma,** derived from cambium-cells by their division by transverse walls.

iv. Some few cells, especially towards the periphery, containing **crystals** which give the reactions of calcium oxalate.

THE LEAF.

Cut transverse sections of a foliage leaf of *Pinus sylvestris,* taken from a stem of the current year. It may be found convenient to embed in paraffin, or to hold the leaf between pieces of pith, or carrot. Mount as before, and examine with a low power. Note the form of the section; the flat side is the upper, the convex side the lower. Observe successively the following tissues:—

A. A single layer of **Epidermal cells** with very thick walls.

B. A narrow band of thick-walled **Hypoderma.**

C. A broad band of chlorophyll-containing **Mesophyll,** with resin-passages.

D. A **Bundle-sheath,** consisting of oval cells.

E. A broad band of tissue without chlorophyll, which surrounds—

F. Two central **Vascular bundles.**

Study these several tissues under a high power.

A. The **Epidermal cells** have their thick walls differentiated into three layers. These may be recognised without staining, or better after treatment with Schulze's solution, as—

i. A thin external **Cuticle,** not very deeply stained. It extends as wedge-like processes between the cells.

ii. The **Cuticularised layers,** forming a thick band, which stains a deep brown. Immediately surrounding the cell-cavity is—

iii. A broad pitted band, not deeply stained.

This differentiation may be brought into greater prominence by treating (*a*) with strong sulphuric acid, or (*b*) by staining slightly with fuchsine. (*c*) If sections are boiled for ten minutes or more in strong solution of potash, i. will be dissolved while ii. and iii. remain.

Note the larger cells at the angles of the section, with thicker walls.

Here and there depressions of the external surface may be observed. These indicate the position of the **Stomata.** Observe the two **guard cells,** which are seated some distance below the surface of the leaf.

B. The **Hypoderma** (sclerenchymatous) varies in thickness from a single layer of cells to several layers. It is thickest at the corners of the section; cells thick-

walled, lignified. Note that it is absent below the stomata.

C. The **Mesophyll** consists of thin-walled, chlorophyll-containing parenchyma. The cellulose walls (blue with Schulze's solution) show a peculiar infolding. **Resin-passages** occur in it. Their cavity is lined with thin-walled epithelium, which is immediately surrounded by a layer of thick-walled sclerenchyma.

D. The **Bundle-sheath,** walls stained brown with Schulze's solution.

E. The tissue within this consists of two elements:

i. **Parenchymatous cells,** with thin cellulose walls (blue with Schulze's solution), and protoplasmic contents.

ii. Elements having lignified walls, with bordered pits, and no cell-contents (tracheides, transfusion-tissue. [Mohl.]).

F. The two central **Vascular bundles,** the constituents of which resemble those of the stem. Note that the xylem is directed towards the upper surface. Thick-walled sclerenchyma is scattered irregularly round the bundles.

THE ROOT.

I. Cut transverse sections of a young primary root of the seedling of *Pinus* (not necessarily *P. sylvestris*); treat with dilute potash, and mount in glycerine. Observe:—

1. A thick band of **Cortex,** not covered externally by any true epidermal layer (*cf.* longitudinal sections of apex of root).

2. A **Bundle-sheath** within the cortex. This is a

single layer of cells, having the characteristic marking on the radial walls. Within this lies—

3. The **Pericambium,** a band three or four layers of cells thick. This immediately surrounds—

4. The central **Vascular cylinder,** in which may be seen—

a. Y-shaped groups of **Xylem** elements, the fork of the Y directed outwards; their number varies (3—6). Between the limbs of the fork of each lies a **resin-passage.**

b. Groups of **Phloem** elements, equal in number to the xylem groups, and alternating with them. N.B.—These tissues of the phloem are not very easily recognised.

c. Centrally is a mass of **parenchyma,** which also extends between the xylem and phloem masses, and separates them from one another.

II. Cut other sections from an older part of the root, and treat as before. Observe that:—

1. The cortex becomes disorganised and brown.

2. Divisions appear in the outermost cells of the pericambium, forming a layer of cork.

3. Lateral roots may occasionally be found, originating in the pericambium, opposite the xylem.

4. The parenchyma lying centrally to the phloem groups has begun to divide as a **Cambium-layer.**

III. Cut transverse sections of a thin lateral root (about $\frac{1}{32}$ of an inch in diameter) of a full-grown tree of *P. sylvestris;* mount some sections in glycerine, others in Schulze's solution. Observe successively, starting from the periphery of the section:

1. Withered remnants of the **Cortex.** This may, however, have been already completely thrown off.

2. The **Pericambium,** with its secondary products arranged thus:—

a. Externally a thin band of **Cork,** the cells of which are arranged in radial rows.

b. The **Cork-cambium,** the cells dividing by tangential walls.

c. The remainder of the original pericambium in a quiescent state.

3. The **Phloem,** forming, according to the age of the root, a more or less complete ring. The constituents resemble those of the phloem of the stem, and are often distorted by external pressure.

4. The **Cambium,** as in the stem.

5. The **Xylem,** in which may be recognised, near the centre—

a. The **primary xylem** groups, arranged in the form of a Y, and each having, as before, a resin-passage in the fork.

b. The masses of **secondary xylem,** more or less fan-shaped, and alternating in position with the groups of primary xylem. The number of the latter, and of the masses of secondary xylem, varies in the lateral root, four being the average number. The constituents of the secondary xylem resemble those of the stem in structure and arrangement.

IV. Cut, and mount as before, transverse sections of a root about one-eighth of an inch in diameter.

The arrangement of tissues will be as before, but the fan-shaped masses of secondary xylem will have joined laterally, so as to form a complete ring. **Annual rings** may also be seen—in fact, the structure at the periphery of the root now closely resembles that of the stem.

V. Cut median longitudinal sections of the apex of the root of *Pinus*. This may be easiest done by cutting longitudinal sections of the mature embryo in the seed. Treat with potash till they are transparent, and mount in glycerine. Observe :—

1. The central **Plerome** cylinder, recognised as in the Sunflower and the Maize. It is rounded off at the apex, and throughout is quite distinct from—

2. The **Periblem,** which surrounds it. This is the formative tissue of the cortex. Outside this no true epidermis is to be found ; but at the apex is—

3. A **Root-cap,** which is formed by the active division of the cells of the periblem at the apex of the root.

Compare this arrangement of the apical meristem with those types seen in the roots of Angiosperms.

REPRODUCTIVE ORGANS.

We have seen at the apex of the ordinary vegetative branch in spring, an apical bud surrounded by a number of lateral buds, all of which normally develop into vegetative axes of the type above described. The **reproductive** organs of *Pinus* are produced on buds corresponding in position to these : they are easily recognised, even at an early stage of development, with the naked eye.

The following observations should be made upon museum specimens, otherwise they could only be made at intervals, according to the period of development of the organs in question.

I. **Male inflorescence.**—A. Note that the inflorescence while young, appears as a bud covered with brown

scale-leaves, in the axils of which are **lateral axes** easily seen on removing the scales. Of these lateral axes—

(*a.*) Those nearest the apex of the bud develop as lateral foliage-shoots (*cf.* ordinary vegetative axis).

(*b.*) Below these, a number bear, in place of the two foliage leaves, numerous **Staminal leaves** (these axes are **Flowers**).

Comparing the male inflorescence with the ordinary vegetative axis, the main difference lies in the mode of development of the lateral axes. In autumn the male inflorescences of the preceding summer can only be distinguished from the purely vegetative axis, by the absence of the lateral foliage-shoots from the lower parts of them.

B. Separate a single male flower, and cut it longitudinally in a median plane: it will be found to consist of—

1. An **Axis,** which bears.
2. A number of **Staminal leaves.**

Detach some of these staminal leaves with a needle: each consists of—

(*a.*) A short stalk, or **Filament,** which bears at its apex—

(*b.*) An expanded **Anther,** with two swellings (**Pollen-sacs**, or **Microsporangia**) on the lower surface.

C. Cut longitudinal sections of the male flower in which the pollen is not yet ripe, and mount in glycerine: examine with low power. Note the arrangement of the parts as above described. In the pollen-sacs note the **Pollen-grains** *in situ* (**Microspores**).

The pollen is ripe about the middle of June, and material should be collected and preserved in alcohol at such time as to

illustrate various stages of development (*i.e.*, at short intervals during May and June). By cutting sections from such material, and treating as above directed, the history of development of the pollen may be made out.

D. Mount ripe pollen-grains (*i.e.* such as may be collected by shaking a male branch in June) in dilute glycerine, having previously wetted them with alcohol. Observe—

1. The two large lateral **Wings,** usually filled with air, which facilitate the carriage of the pollen by the wind. These are extensions of the outer coat (extine).

2. The central body of the pollen-grain consisting of—

(*a.*) A large cell, which constitutes the greater part of the grain, and from which the pollen-tube springs.

(*b.*) A series of one or more smaller vegetative cells, affixed laterally to the wall of the pollen-grain at a point between the wings. These take no direct part in the formation of the pollen-tube.

II. **Female branches or Cones.**—Observe on a Scotch Fir, towards the end of June, that there are cones to be found in three different stages of development, the position of which is constant.

(*a.*) **Small green cones** occurring (one or more) close to the apex of the shoot of the current year. Note that the basal part, or stalk, bears brown membranous scales, while the upper part is globular, and is marked out into numerous square areas, which are the apices of so many **Ovuliferous scales.**

Comparing a shoot, which bears such young cones, with an ordinary vegetative shoot, it will be seen that the cones correspond in position to the lateral buds, of which they are the morphological equivalent.

(*b*.) **Larger green succulent cones,** which occur laterally at the apical part of the shoot of the previous year: the arrangement of parts on these corresponds to that on (*a*).

(*c*.) Cones larger than (*b*), brown and with lignified tissues: on these the scales are usually more or less separated from one another so as to disclose the **seeds,** two of which are borne at the base of each of the ovuliferous scales. These ripe cones are seated laterally near the apex of the two-years-old shoot.

A. Cut median longitudinal sections of a cone corresponding to stage (*a*). It should previously have been hardened with alcohol for some days: mount in glycerine, and examine with a low power. Observe—

1. The central **axis,** not differing essentially from the young vegetative axis: on this are borne scales of two orders easily distinguished by their size.

2. The **smaller** of these are the leaves borne by the axis of the cone, and the morphological equivalents of the brown scale-leaves which cover the winter buds. In the axil of each of these is borne one of—

3. The **larger** or **Ovuliferous scales,** which are longer and more bulky than (*b*): they alone can be seen externally. On the upper surface of each of these, close to the axis, are borne—

4. Two **Ovules,** or **Macrosporangia,** which are **anatropous,** so that the micropyle is directed towards the base of the scale: if cut in a median plane, each ovule will be seen to consist of—

i. One **Integument,** several layers of cells thick, with a widely open **Micropyle** facing the axis; this is—

ii. The **Nucellus,** a mass of parenchyma, near the centre of which is—

iii. The **Embryo-sac** or **Macrospore,** a cell much larger than those of the surrounding tissue and lying some distance below the apex of the nucellus.

Pollen-grains may often be found seated on the apex of the nucellus, one or more of these may throw out **Pollen-tubes,** which penetrate into its tissue.

Dissect off one whole **Ovuliferous scale,** and observe on its upper surface, close to the base, **two Ovules,** which are anatropous. Note also the relative positions of the two sets of scales.

B. Take cones of the stage above described as (*b*).

The materials should be collected about the middle of June, and must be hardened in alcohol.

Strip off the ovuliferous scales of such cones. The ovules will remain adherent to the base of each. Cut longitudinal sections of the scales so as to pass through the median planes of the ovules; mount in pure glycerine, and examine with a low power. Observe—

1. The structure of the **Ovuliferous scale,** which is traversed by vascular bundles, and resin-passages.

2. The **Ovule,** which is united with the scale, and consists, as in the younger stage, of—

a. An external **Integument.** Note the wide **micropyle;**

b. The **Nucellus;**

c. The **Embryo-sac** filled with **Endosperm.** All the parts of the ovule are larger than in the younger stage, but retain the same relative positions. Note carefully that **Pollen-grains** (one or more) are usually

to be found lying on the apex of the nucellus; and that from the larger cell of each of them arises a **Pollen-tube,** which traverses the tissue of the nucellus, as far as the apex of the endosperm, where it widens out into a large sac.

Observe near the apex of the endosperm, and embedded in it, one or more large vacuolated protoplasmic bodies; these are the **Egg-cells,** or **Oospheres.** From the apex of each may be traced a narrow **neck** or channel, inclosed by smaller cells than those of the surrounding endosperm. The neck and central cell together form the **corpusculum** (that is the **archegonium**).

C. Remove ovules from cones of the second year taken and preserved in alcohol about August 1. Dissect off from them the now hardened **Integument** (**Testa**). Note within this the delicate remnant of the **Nucellus,** which covers the mass of **Endosperm.** Soak the latter in water, and dissect from it with needles the **Embryos** (numerous), which lie in the central cavity of the endosperm; treat them with potash, and mount in dilute glycerine. Examine with a low power, and observe—

1. The **Suspensors,** coiled filaments consisting of numerous transparent thin-walled cells. At the ends of the suspensors are borne—

2. The **Embryos;** they are more or less elongated, almost cylindrical bodies; in some cases (only one as a rule in each seed) they may have already formed—

a. An **Apical cone,** which terminates the free, anterior end of the embryo; this being surrounded by—

b. A whorl of **Cotyledons** of variable number.

c. The apex of the **Radicle,** directed towards the suspensor (*i.e.* towards the micropyle of the ovule), and embedded in the tissue at the posterior end of the embryo.

Note that there is no definite boundary between the suspensor and the embryo.

Also that though **polyembryony** is the rule, *i.e.* a number of embryos are at first formed simultaneously, one of these supersedes the rest, and that one alone becomes differentiated as above described.

By comparing sections of ovules of various ages (*i.e.*, taken between the dates above named), cut and treated in the manner described for the cones taken in June [p. 158], the history of the early stages of development of the suspensors and embryos from the fertilised egg-cell may be traced.

Ripe Seed.

Examine the ripe seed of *P. sylvestris,* or other species *e.g. P. pinea ;* and note the external hard and thick **Testa ;** within this the **Endosperm,** which incloses the single **Embryo.** It has numerous **Cotyledons** and **Radicle,** the apex of the latter being directed towards the micropyle.

Germination.

Compare plants in different stages of germination, and observe the following points in the process :—

1. The endosperm swells, and bursts the testa.
2. The radicle protrudes, and curves downwards.
3. The cotyledons elongate, and push out the stem, and their own basal portion.

4. The seed is usually carried upwards on the apex of the cotyledons, which, with the hypocotyledonary stem elongate greatly.

5. The plumule develops, forming numerous acicular leaves.

N.B. The cotyledons turn green while still protected from the light, below the soil, and within the testa.

PTERIDOPHYTA.

A.—LYCOPODINÆ.

I.

SELAGINELLA MARTENSII.

SPOROPHORE.

I. In a well-grown plant note with the naked eye the following external characters:—

(1.) The **Stem** ascending, frequently branched, apparently in a dichotomous, but really in a monopodial manner (see below); the branching occurs only in a single plane.

(2.) The **Leaves** simple in form, with a ciliate margin, and arranged in alternating pairs; each pair consists of a dorsal and a ventral leaf, the whole series thus forming **four orthostichies:** note the two different sizes of leaves—

a. The **larger ventral** leaves, arranged in two orthostichies, without terminal awns.

b. The **smaller dorsal** leaves, also arranged in two orthostichies, each leaf being terminated by a fine **Awn.**

Each leaf has a single central nerve or **Midrib.** Turn back one of the leaves, and observe with a lens

the small scale-like **Ligule.** Note that the insertion of each leaf is oblique.

(3.) The **Rhizophores,** long cyclindrical branched organs, which arise at the points of branching of the obliquely ascending stem, and grow vertically downwards: note their frequent bifurcations.

N.B. Two rhizophores are formed at each branching of the axis, one on the dorsal, and the other on the ventral side; of these only the latter is developed beyond the first rudimentary stage.

Remove carefully a rhizophore, which has grown down so as to reach the soil, and wash it: observe—

(4.) The delicate bifurcating **Roots,** which rise at the point where the rhizophore touched the soil.

Observe further that many of the branches of the stem may have a symmetrical arrangement of the leaves close to the apex: these are the branches or **Cones,** which bear the **Sporangia:** note that on these cones—

(i.) The leaves are all similar to one another and of small size.

(ii.) That they are arranged in four symmetrical orthostichies.

(iii.) That, on turning the leaves back, one **Sporangium** will be disclosed in each case. On comparing a number of sporangia, which have been exposed in this way, it may be seen that there are two sorts of them—

(*a.*) **Macrosporangia,** which are of a green or light-brown colour, and appear to be of rounded tetrahedral form.

(*b.*) **Microsporangia,** which are more nearly spherical, and of a reddish-brown colour.

Note in older cones that the sporangia are already

open, **dehiscence** having taken place in a plane parallel to that of the leaf.

II. Cut out as thick a piece of the stem as can be found, and about one inch in length: note on the surface of transverse section a central white dot; this is the single central **vascular bundle.** Slice off the upper surface of the stem with a razor till the whole course of the vascular bundle is laid bare, and observe with a lens—

1. The course of the central vascular bundle, which is directly longitudinal and median.

2. The smaller lateral bundles, which pass from the central bundle without branching, into the leaves, and traverse the midribs of the leaves.

III. Cut transverse sections of a well developed stem: mount some in glycerine, others in Schulze's solution (others again may be mounted in acid solution of aniline sulphate). Examine first under a low power, using a high power when necessary, and observe the following tissues in succession, starting from the periphery of the section:—

1. At the periphery a layer of small, thick-walled cells, forming an ill-defined **Epidermis,** with no stomata; it is covered externally by a continuous **Cuticle.** Beneath the epidermis, and not clearly marked off from it, is—

2. The **Cortical tissue:** the cells of the peripheral part of it have thick, stratified, and lignified walls, with no intercellular spaces. Passing inwards there is seen a gradual decrease in thickness of the walls, and increase in size of the cells, till an abrupt limit is reached at—

3. The **Lacunar tissue,** consisting of thin-walled cells, which form irregular **trabeculæ** traversing the intercellular cavity in a radial direction: the inmost cells of these trabeculæ have a peculiar equatorial constriction.

This lacunar tissue is more typically represented in some of the larger species, *e.g. S. inæqualifolia, S. Willdonovii,* &c. It may be regarded as the equivalent of the **bundle-sheath** of most other vascular Cryptogams.

4. By means of these trabeculæ the single central **Vascular bundle** is suspended in the middle of the large air-cavity: the bundle is built upon the **concentric** type, and is composed of the following tissues:—

a. The **Phloem-sheath,** an irregular band of comparatively large, thin-walled cells, which completely surround the central tissues, and abut externally on the intercellular cavity, and the trabeculæ.

N.B. The cells of this layer, in common with all the outer tissues, including the epidermis, may contain chlorophyll granules, which are large, and only few are to be found in each cell.

b. The **Phloem,** recognised as a tissue with thin cellulose walls, small cavities, and sparing protoplasmic contents; it forms a continuous band surrounding—

c. The central **Xylem,** which appears as a spindle-shaped mass of tissue when seen in transverse section, and consists of elements with lignified walls, and no cell-contents.

N.B. Small vascular bundles of rounded outline, as seen in the transverse section, may be found opposite or

near to the ends of the spindle-like vascular bundle; these are bundles of the leaf-trace cut through on their course inwards from the leaves. Thus the whole bundle-system of this shoot consists of a single central bundle, which traverses the axis longitudinally, and gives off smaller branch bundles, which pass outwards into the leaves, one of them entering each leaf.

The vascular system of the shoot is more complicated in certain other species of *Selaginella;* thus in *S. Willdonovii*, *S. inæqualifolia*, &c., three large flattened bundles are seen in each transverse section of the axis; the planes in which these bundles are flattened are parallel to one another; in these species the bundles may individually show considerable irregularities of outline: the whole arrangement of their vascular system may with advantage be compared with that in the stem of *Lycopodium*.

Note with a higher power:—

1. The general appearance of the **Phloem,** with its highly refractive cellulose walls, and scanty protoplasm.

2. Between this and the xylem a somewhat irregular series of cells of the **Conjunctive parenchyma,**[1] with thin cellulose walls and plentiful protoplasm.

3. The chief constituents of the **Xylem,** viz., large prismatic **Tracheides,** with peculiarly marked, lignified walls.

4. At the poles of the spindle-shaped xylem note tracheides of smaller size; these compose the first formed **Protoxylem:** development thus proceeds from the periphery to the centre.

[1] It has been suggested by Treub that this term, which has been applied to the central parenchyma of certain roots, may be extended so as to include that parenchymatous tissue which has been termed by Russow "Geleitzellen."

To confirm this, cut transverse sections of the stem about one inch from the apex, and treat as above. It will be seen that the elements near the poles of the xylem are already fully formed, and their walls lignified, while those at the centre are still thin-walled, and have protoplasmic contents. The lacunar tissue will be found to be better defined in these sections.

IV. Cut longitudinal sections of a stem of *S. Martensii.*

N.B. Since, owing to its being fixed in its place only by the weak trabeculæ, the vascular bundle is apt to be detached in cutting accurately longitudinal sections, it will be found better to cut the sections slightly oblique; it must then be remembered in examining them under the microscope that the sections are not truly longitudinal.

Mount some in glycerine, others in Schulze's solution, and examine them under a high power, noting the same succession of tissues on starting from the outside, as were seen in the transverse sections: thus —

1. **Epidermis** } these are hardly to be distin-
2. **Outer cortex** } guished one from another; the cells of both are prosenchymatous, and thick walled, and show a gradual transition to—

3. The **Inner cortex** with thinner walls, and of parenchymatous shape.

4. The **Lacunar tissue,** in which may be distinguished—

a. The outer parenchyma, consisting of short and small cells.

b. The inner cells, which are elongated in a radial direction, and show the peculiar median constriction, above noted in the transverse sections.

5. The **Phloem-sheath,** consisting of elongated

parenchymatous cells, with cellulose walls, and often containing chlorophyll.

6. The **Phloem,** the most prominent elements of which are long narrow structures with cellulose walls and sparing contents: these are regarded as the representatives of the **Sieve-tubes.**

7. The **Xylem,** the most prominent elements of which are **spiral** and **scalariform Tracheides,** similar to those composing the xylem of the bundle in the Ferns (see below, p. 195). The walls are lignified and thickened, and marked by elongated pits, which by their regular arrangement give the scalariform character to these elements.

V. Cut transverse sections of a **Rhizophore,** mount as before, and observe that (1) the peripheral tissues are not unlike those of the stem, and are marked off from the central cylinder by a somewhat irregular **Bundle-sheath:** (2) that the arrangement of tissues of the central cylinder differs both from that of the stem, and that usual for root-structures, there being but one group of **Protoxylem** (monarch), which is placed laterally, and the later formed **Xylem** forming together with it a central mass, which is surrounded by **Phloem** except at the point opposite the protoxylem. The structure of the individual vascular elements is similar to that in the stem.

If successful median longitudinal sections be cut through the apex of a rhizophore it will be found that there is no root-cap. Further, by comparison of a number of sections, both longitudinal and transverse, it may be concluded that there is one **apical cell** having approximately the form of a quadrangular pyramid.

VI. Cut transverse sections of a **Root,** and mount

as before: the structure will be found to resemble that of the rhizophore.

If median longitudinal sections be cut through the apex of a root, a **root-cap** will be seen, which covers an **apical cell**, having the form of a triangular pyramid.

VII. Mount some leaves, both dorsal and ventral, which have been previously bleached in alcohol, in water, or dilute glycerine: examine with a low power, and observe:—

1. The difference in form of the dorsal and ventral leaves.
2. The central **Midrib.**
3. The **ciliate margin.**
4. The characters of the **Epidermis;** thus on—

a. The **upper surface** the cells are small, and circular in surface view, with sinuous lateral walls, and **no stomata;**

b. The cells of the **lower surface** are elongated, with pointed ends, and sinuous lateral walls; over the midrib the cells are shorter, and it is there only that the **Stomata** are found, having two guard-cells, and no subsidiary cells.

Large chlorophyll grains are to be found in the cells of both the upper and the lower epidermis, and in these the included starch-grains may often be well seen after treatment with iodine.

VIII. Cut transverse sections of fresh leaves held in a piece of pith: mount in water or weak glycerine, and observe:—

1. The **Epidermis** of the upper surface (without

stomata) consists of conical cells, with few, very large chlorophyll granules.

2. Beneath this is the **Spongy parenchyma,** which encloses centrally—

3. A single **Vascular bundle.**

4. The **Epidermis** of the lower surface consists of smaller cells containing chlorophyll, and with **Stomata** opposite the midrib: note the two small guard-cells as seen in transverse section. Near the margin of the leaf the upper and lower epidermal layers are in contact with one another, the spongy parenchyma being there absent. There is also a marginal band of thickened cells.

IX. Choose out from material which has been hardened in alcohol the **apical buds** of branches which have not as yet begun to form sporangia: holding these between pieces of pith, or carrot, or otherwise embedding them, cut longitudinal sections; mount them in glycerine, examine first with a low power, and select those sections which are nearest to the median plane (*i.e.* those which show the greatest regularity of parts, and the stem terminated by the apical cone).

In such sections observe :—

1. The **Axis** with tissues as above described (*cf.* longitudinal sections), and terminated by the apical cone: borne laterally on this are—

2. The **Leaves,** each having a **Ligule** attached to its upper surface: note also their structure as above described. Passing towards the apex of the bud observe successively earlier stages of their development.

Examine the sections with a higher power, and observe :—

1. The arrangement of the cells at the summit of the apical cone, which is terminated by an **apical cell**; from this segmental cells are successively cut off.

2. The origin of the leaves is not from a single cell, but by the outgrowth and subsequent division of a number of cells at the periphery of the apical cone.

In such median longitudinal sections should also be observed the differentiation of the vascular bundle from the primary meristem, and also the development of the lacunar tissue, and its relation to the central bundle.

Preparations may also be made of the apex so as to show the structure of the apical cone as seen from above. By comparison of a number of these it may be seen that the form of the apical cell is by no means constant, but varies between the forms of a two-sided and a three-sided cone.

X. Cut longitudinal sections through fertile branches similar to those cut from the vegetative bud, and examine them under a low power.

Observe that the general arrangement of the stem, leaves, and ligules is the same as in the vegetative bud. In the lower part of the sections a mature **Sporangium** may be found in the axil of each leaf. (The sporangium may have lost its spores partially or entirely during the preparation of the sections.) It will consist of—

(*a.*) A short massive **Stalk.**

(*b.*) A **Wall** enclosing the central cavity: the wall will be found under a high power to consist of three layers of cells—

i. The outer consisting of thick-walled cells, more or less elongated radially.

ii. A layer of small, compressed cells.

iii. A layer of thin-walled cells, elongated radially: this is the **Tapetum,** which is here persistent until the spores are ripe.

Surrounded by the wall will be found—

(*c.*) **Spores** of two sorts—

i. **Microspores** of relatively small size; these will be found in large numbers in certain sporangia, which will accordingly be recognised as **Microsporangia.** When ripe they may be still seen to cohere in groups of four: each spore is a single cell with a brown wall.

ii. **Macrospores** of relatively large size: **four** only of these will be found enclosed in the sporangium, which is accordingly termed a **Macrosporangium.** Each spore consists of a thick wall, with numerous external projections, surrounding a large cavity filled with protoplasm.

The development of the sporangium may be traced in longitudinal sections of sporangium-forming cones which have been hardened in alcohol, or better, in picric acid and then in alcohol; mount in glycerine. The following points in the process of development may be observed. The sporangium is first seen as a swelling of a group of cells at the surface of the apical cone, above the leaf in the axil of which it appears: thus the sporangium is not borne on the leaf as in *Lycopodium*, but springs from the tissues of the axis. A central row of cells grows more strongly than the rest, and the outermost cell but one of this series may be recognised as the **Archesporium.** The outermost cell divides to form part of the two outer layers of the wall of the sporangium. The archesporium also divides to form a mass of tissue, of which the peripheral layer becomes the **Tapetum** (the basal part of the tapetum is however derived from the adjoining tissue). The central part of the tissue derived from the archesporium forms the spores; each spore-mother-cell separates from its neighbours, and divides into **four** cells. If the spo-

rangium is to develop **macrospores,** only one of these groups of four cells is further developed, the rest being abortive; if it develop **microspores,** all the groups of four are further developed, but only attain a comparatively small size: in both cases the four spores may separate from one another when quite mature, though they often retain their original arrangement.

THE OOPHORE.

XI. Spores of both kinds may be obtained free by drying branches which bear sporangia on sheets of paper. Pick out the **macrospores,** and mount them in olive oil; dissect off the brittle outer coat of the spore with needles, and examine under a high power. It will be seen that the chief contents of the ripe spore are a protoplasmic matrix enclosing oil globules and aleurone grains, while traces of the cells of the **Prothallium** may be recognised even in these preparations.

Prepare other such spores with potash, and dissect as before, or press on the cover slip, and warm gently. It will be found, when the oil, &c., has been acted upon by the potash, that a part of the contents of the spore is traversed by a distinct network of cell-walls, forming a meniscus-shaped mass of tissue. If plenty of spores are to be had, it will be found better to embed a quantity of them in cocoa-butter, and to cut sections, and mount them in glycerine. Observe—

1. The character of the wall, consisting of—

a. An outer thick, yellow **Exospore.**

b. An inner thin **Endospore.**

2. The contents as above described: the natural position of the cellular tissue of the **Prothallium** may be seen to be at the apex of the cavity of the spore.

XII. Spores of both kinds should be collected in considerable quantity by drying on paper, and then be sown on moist soil or sand, and left to germinate. In a few weeks young seedlings will be seen with an erect axis, bearing small leaves. The axis branches at an early period.

Remove one of these seedlings from the soil, and note the bifurcations of the root, and the macrospore still attached laterally to the axis.

By careful comparison of spores thus sown, it may be observed at various times during their development that the contents of the microspores divide into a number of cells, and ultimately rupture and set free **antherozoids**: also that the tissue of the **prothallus** in the macrospore increases, rupturing the wall of the spore, that **archegonia** are formed, from one of which the young seedling originates: for further details the Text-books must be consulted.

II.

LYCOPODIUM CLAVATUM (The Common Club-Moss).

SPOROPHORE.

I. In a well-grown plant recognise the following external characters :—

1. The **Stem,** often extended to a great length, is creeping, and frequently branched, apparently in a monopodial manner (for particulars see below): the stronger branches are also creeping, the weaker branches ascending.

2. From the under side of the stem **Roots** are developed, which frequently (but not always) appear at points where the stem branches. The roots themselves are branched dichotomously, but the limbs of the dichotomy may develop either equally or unequally.

3. The stem is covered with **Leaves,** which are simple in form and linear, with ciliate margin, and a long awn-like apex. The arrangement of the leaves is complicated, and has been described by Braun as being partly in whorls, partly spiral: the number of members of the whorls is variable, as is also the angle of divergence of the spirally-arranged leaves.

4. The fertile branches, or **Cones,** which bear

sporangia, are erect and elongated; their lower part is covered sparsely with leaves of small size: about 1—2 inches below the apex they usually divide into two or three branches, covered with rather broader, closely imbricated leaves: from the upper surface of each of these rises one **Sporangium,** which is yellow when ripe, and opens by a split parallel to the plane of the leaf which bears it.

II. Cut transverse sections of a fully developed stem. Mount some of them in glycerine, others in Schulze's solution, and examine with a low power: externally will be found—

1. An **Epidermis,** consisting of a single layer of cells; their outer walls are thick, and covered by a continuous layer of **Cuticle,** which may be recognised in thin sections by its high refractive power.

2. Below the epidermis lies a broad band of **Cortical tissue,** which appears differentiated into successive thinner bands according to the thickness of the cell-walls: thus there may be distinguished—

a. An external **sclerenchymatous** band, with thick lignified walls (brown with Schulze's solution); small intercellular spaces may be seen at the angles between the cells: these cells retain a small proportion of their cell-contents.

b. Within this is a broad band of **thin-walled tissue,** in which the cell-contents are not apparent: the cell-walls are tinged with pink in Schulze's solution. There is a gradual transition from this to—

c. The most central part of the cortex, which is strongly **sclerenchymatous;** it has intercellular spaces, and retains its cell-contents. It forms a dense

band of lignified tissue (brown with Schulze's solution). Here and there may be found in the cortex small groups of very narrow elements, having a dark appearance: these are single **Vascular bundles of the leaf-trace**, cut through on their course from the leaves to the central vascular cylinder. A sudden transition is found from the inner sclerenchymatous band (*c*) of the cortex to—

3. The tissue, which lies next to it internally. This tissue consists of two to three layers of tangentially elongated cells, the walls of which have a sharp contour, are not thick, and stain with Schulze's solution a slightly different tint of brown from the walls of the sclerenchyma: this band is regarded as taking the place of the **Bundle-sheath**, which is met with in most of the Pteridophyta as a definite layer of cells.

Treat a section with sulphuric acid: the walls of this band will be found to resist its action more than the rest of the tissues, the walls being of a corky nature. Note also that the cuticle is brought into greater prominence by treatment with sulphuric acid, since it resists the action more strongly than the other walls.

Within this so-called bundle-sheath is found—

4. A cylindrical mass of **vascular tissue.** It is composed, as in other cases, of (*a*) **Phloem tissues,** and (*b*) **Xylem tissues,** which may be distinguished by their optical properties, and by their staining with various reagents. Observe that the phloem forms a matrix, as it were, in which are embedded the masses of xylem; the latter are of elliptical form as seen in the transverse section: several of these

are ranged side by side, the longer axes of the ellipses being horizontal as the plant grows, and parallel to one another: the masses of xylem may be irregularly connected one with another towards the centre of the stem.

III. Before proceeding to the study of these several tissues in detail, cut transverse sections from the young stem, at about one eighth of an inch below the apex; treat some of these with dilute potash, others with Schulze's solution. Observe that in these sections the tissues at the centre of the vascular cylinder are still thin-walled, and have plentiful protoplasm, *i.e.*, they are not fully developed; towards the periphery, however, will be seen a series of groups of tissue showing the characters of developed xylem, and alternating with these is a series of groups of phloem tissues. Other preparations may be made successively from points further from the apex, and from these the conclusion may be drawn that the vascular tissues at the periphery of the vascular cylinder are matured first, and that the development proceeds towards the centre. (Compare roots.)

IV. Returning to the sections of the old and mature stem, examine the vascular tissues under a high power.

i. Immediately within the so-called **Bundle-sheath** is a band of tissue, which abuts directly upon the periphery of the xylem and phloem, and having cell-walls which stain blue with Schulze's solution; this may be regarded as the **Phloem-sheath.**

ii. On examining the masses of **Xylem,** observe that—

a. The constituent elements are much smaller at the periphery of the vascular cylinder than towards the centre; the former are the first developed or **Proto-xylem** elements.

b. The main constituents of the xylem are elements with large cavity, and of rounded polygonal form

(**Tracheides**); note the structure of the wall, especially where two of these adjoin one another.

iii. In the **phloem** there will be found at the periphery of the vascular cylinder, and alternating between the successive groups of protoxylem, masses of tissue with thick cellulose walls, and small cell-cavities; these are the **Protophloem** groups, or first formed elements of the phloem. Passing from these towards the centre of the vascular cylinder, the phloem is found to consist of—

a. Constituents with large cavities, and very scanty cell-contents.

b. Elements with small cavity, and obvious cell-contents.

V. Cut radial longitudinal sections through a mature stem: mount as before, examine them first with a low power, and note—

1. The bases of the leaves continuous with the epidermis and cortex of the stem.

2. The **Cortex** showing the same differentiation into successive bands (*a*), (*b*), (*c*), as was seen in the transverse section.

3. The central **Vascular cylinder.**

4. Small **Vascular bundles of the leaf-trace,** which may be seen pursuing an oblique downward course from the bases of the leaves, through the cortex to the periphery of the vascular cylinder; since these bundles may be followed in one radial section from the leaf to the central cylinder, it follows that that part of their course is approximately in a radial plane.

We can now obtain a clear idea of the **Vascular System** of a mature shoot of *Lycopodium clavatum:*

there is in the first place a central vascular cylinder, which traverses the shoot longitudinally, and from the periphery of it single bundles of small size are given off, which take an obliquely ascending course in radial planes, and each of them enters a leaf. By further comparison of longitudinal and transverse sections it may be ascertained that the masses of xylem in the central cylinder have the form of flattened plates, the planes of which are approximately horizontal in the living plant. They are sometimes separate from one another, sometimes joined towards the centre of the stem, and it is on the margins of these plates that the bundles of the leaf-trace are inserted.

Examine the radial longitudinal sections under a high power, and observe—

i. That the cells of the epidermis, and of the sclerenchymatous portion of the cortex are elongated and **prosenchymatous,** while those of the thin-walled band of the cortex are shorter, and tend to a **parenchymatous** form. The walls of the cortical cells are pitted.

ii. Of the vascular tissues the **Xylem** is the most prominent; its chief constituents are of prosenchymatous form, with lignified walls: the latter show the **scalariform** marking, which is due to the regular arrangement of **elongated pits** with their longer axes placed horizontally: each of these pits shows a double contour, and transitional forms will be found from the elongated to circular pits, the latter presenting an appearance very similar to that of the **bordered pits** of *Pinus* from which the elongated pits differ only in their outline as seen in surface view. Some of the

walls will have been cut through longitudinally: examine a section of one of them, and note especially that the **pit-membrane** is constantly present; there is thus no communication between the cavities of these elements, and they have no cell-contents remaining; they are therefore **scalariform tracheides** (compare those in the xylem of the Fern, and the tracheides of *Pinus*.)

iii. Where the sections have passed through the peripheral margins of the plates of xylem, there will be found elements of the **Protoxylem,** which correspond in structure to those in the stem of *Pinus*: irregular finger-like outgrowths of the cell-wall may be observed extending into the cavity of these elements.

iv. The phloem, intervening between the masses of xylem consists of—

a. Prosenchymatous cells with cellulose walls, and granular cell-contents; these are directly in contact with the xylem.

b. Long tubular structures, the pointed endings of which are very rarely met with; their course may be followed for a considerable distance in a longitudinal direction; they have transparent contents, and their cellulose walls are dotted with minute pits, about which bright globules adhere. These are probably the representatives of the **Sieve-tubes** of the Phanerogams.

Stems of other species of *Lycopodium* may be treated in the same way, and a comparison made of their structure; the general arrangement of tissues will be found to be fundamentally the same as that described for *Lycopodium clavatum*, the differences depending chiefly upon the number of plates of xylem and phloem, and variations in the manner and extent of the connection between the plates of xylem.

VI. Cut transverse sections of the leaf; this may easily be done either by embedding the whole stem with the leaves attached in paraffin or cocoa-butter, cutting transverse section of the whole, and then picking out the sections of the leaves; or by holding the stem with the leaves between the finger and thumb, and cutting transverse sections from the whole as from a solid mass. Mount as before; examine under a low power, and note—

1. The outline of the sections roughly triangular.

2. The single layer of **Epidermis** with cuticularised outer wall: **Stomata** are found both on the lower and the upper surface.

3. Beneath this is the **Mesophyll,** with large intercellular spaces: the cells, which form an irregular network, are nearly globular, have thin walls, and contain chlorophyll granules.

4. At the centre is a single very small **Vascular bundle.**

VII. Cut median longitudinal sections through the bud: use material which has been preserved in alcohol, or hardened in picric acid and then in alcohol. Mount in glycerine, and examine with a low power: note that at the lower part of the section the central vascular cylinder will be easily recognised, while the bundles from successive leaves pass obliquely through the cortex, and insert themselves upon its margin. Passing upwards, however, towards the apex, it gradually loses its dark appearance (due to developed xylem); still its continuity may be traced up to the apical cone, as a bright-looking strand of formative tissue consisting chiefly of prosenchymatous

elements (**Plerome** or **Procambium**), while the formative tissue of the cortex external to it is more typically parenchymatous (**Periblem**); it is limited by a not very definite layer of cells which may be recognised as the **Dermatogen.**

The conical apex itself consists of a dome-shaped mass of meristem; the layer of dermatogen, which may be recognised at the base of the cone, may be followed up nearly to the apex, but there loses its identity, the extreme apex being occupied, at least in the more bulky examples, by a group of initial cells, which divide by anticlinal walls; those at the margin of the group divide also periclinally. Compare this on the one hand with the structure of the apex of the stem of Phanerogams, and on the other with that seen in the Ferns.

Observe further that the **development of the leaves** begins by the outgrowth and division (both anticlinal and periclinal) of groups of cells, which constitute multicellular protuberances; these have at first an apical growth, which soon ceases, the further growth being basal and intercalary.

The origin of the **branches** may further be observed in these preparations; it will be seen that they arise in this species **below** the apex of the main axis, and laterally upon it; the branching is thus **monopodial** not dichotomous.

VIII. Cut transverse sections of one of the thick roots: mount as before, and observe that they resemble the transverse sections of the stem in the arrangement of the tissues, though the whole structure is simpler: there are usually only three plates of

xylem; these are often complicated by irregularities, *e.g.*, fusion, &c., and are less strongly developed in their central portion than those of the stem.

If similar sections be cut successively from roots of higher order, they will be found to show successively reduced types of structure, till the xylem is finally represented only by a single group of elements, which is surrounded by tissues of the phloem.

Median longitudinal sections may be cut through the apices of roots which have been hardened in alcohol: from these it will be seen that there is a stratified structure of the apical meristem, in which may be recognised a distinct **root-cap**, marked off from the layers below it by a layer of **dermatogen**, which can be traced as a continuous layer for a considerable distance beneath the root-cap: centrally a strand of **plerome** may be recognised. Bifurcations may be found in such sections, showing that the branching is a true **dichotomy.**

IX. Cut median longitudinal sections through a cone, bearing **Mature sporangia:** mount in glycerine, and examine under a low power; observe the structure of the axis as before seen in longitudinal section, with a vascular system sending out branches into the leaves; the chief difference between this and the vegetative axes is the presence of **sporangia.** Note—

i. That one sporangium is seated with a short stalk on the upper surface of each leaf.

ii. That no branch of the vascular system enters the stalk of the sporangium.

iii. That the cavity of the sporangium is surrounded by a thin wall.

iv. That the cavity thus inclosed may be filled with small tetrahedral spores.

N.B. In preparing the sections the spores are often washed out from the sporangia.

Examine a good section of a sporangium under a high power; the wall will then be seen to be of approximately uniform thickness throughout, and consists of—

a. A well-marked outer layer of cells of considerable size.

b. An inner ill-defined band, consisting of the remains of disorganised cells.

If the wall be observed in surface view the cells will be seen to be of sinuous outline, and somewhat elongated, with the exception of a zone which indicates the line of ultimate dehiscence of the sporangium: here the cells are shorter, and the walls are straight. The line of dehiscence may also be recognised in the wall as seen in section. Note also the structure of the **Spores**; they have the form of a rounded tetrahedron, and the outer wall is covered with peg-like projections.

By cutting similar median longitudinal sections of cones in various stages of development, and comparing them, the history of development of the sporangium may be traced. It may be seen that the sporangium arises as a multicellular outgrowth of the upper surface of the leaf; at an early stage the **archesporium** may be recognised as a hypodermal cell, or possibly several cells: the superficial layer of cells above it gives rise by division to three layers; of these the innermost is the **tapetum,** which, together with the next outer layer, is disorganised as development proceeds, while the outermost layer is still persistent in the mature sporangium. The archesporium meanwhile divides to form numerous **spore-mother-cells,** each of which divides tetrahedrally, and gives rise to four spores.

The proper conditions for germination of the spores of our native species not having as yet been discovered, reference should be made to the Text-books for further information as to the life-history of Lycopodium.

B.—FILICINEÆ.

ASPIDIUM FILIX-MAS (The Male Shield Fern).

A.—MATURE SPOROPHORE.

I.—External Characters.

I. Taking a well-grown plant of the common Male Fern in summer, wash the soil away from the roots, and observe the following external characters :—

A. The **Stem** is oblique and ascending. It is not branched at its apex ; its surface is covered by the persistent bases of the **Leaves,** which are densely covered by numerous brown scaly hairs (**Ramenta**).

B. The **Leaves,** the most prominent of which are—

i. The fully-developed green leaves of the current year ; these are large and of complicated structure, and the following parts may be recognised :—

a. A long almost cylindrical leaf-stalk which is traversed by two longitudinal, lateral ridges or reduced wings. This leaf-stalk supports—

b. The numerous **Pinnæ,** which are arranged in two lateral rows, corresponding in position to the lateral ridges above mentioned. Note that the arrangement of the nerves in the segments of the pinnæ is based upon repeated bifurcation of the stronger nerves. On the under side of the pinnæ will frequently be found—

c. **Sori,** which are roundish groups of small stalked bodies (**Sporangia**), covered by a kidney-shaped **Indusium.**

ii. The bases of the leaves of previous years will be seen, covering the lower part of the stock or stem externally. Observe that lateral **buds** are frequently to be found connected with these, being attached to their posterior side, near to their point of junction with the stem.

iii. Nearer the apex of the stem than the expanded leaves of the current year, and completely covering it, are **young leaves,** densely covered with brown scales (**Ramenta**); these, together with the axis, constitute the **Apical bud.** Note that the apex of each such leaf is rolled up like a crozier (**circinate vernation**).

N.B. Here, as in most Ferns the development of the leaves is very slow; the young leaves seated round the apex represent the foliage leaves of the two succeeding years.

C. The **Roots** are rather thin and brown, with transparent apices: they are inserted **on the bases of the leaves,** close to their junction with the stem: the branching of the roots is monopodial, and the branches appear in acropetal succession.

The stem of *Aspidium Filix-Mas* does not branch at its apex: the same is as a rule the case in the erect stems of Ferns (*e.g.*, Tree Ferns) where the leaves are closely crowded. In those Ferns in which the axis is elongated, a terminal branching is more frequent: thus in *Pteris aquilina* there is a dichotomous branching. In other forms the new axes appear in connection with the leaves, either at the base of the leaf (*Aspidium Filix-Mas*), or in various positions on the flattened upper part of the leaf (many species of *Asplenium*).

II.—*Anatomical Characters to be observed with the naked eye.*

II. Having observed the above external characters, remove the roots, keeping the transparent apices of the young roots, as well as the thickest parts of the old roots: these should be preserved in alcohol for further treatment.

Starting from the posterior end of the stock, cut off successively the persistent bases of the old leaves about ½ inch above their insertion on the stem. Observe the lateral bud borne by some of the leaves on the posterior side of the leaf-stalk near its base: observe also that the roots spring from the bases of the leaves, close to their insertion on the stem.

Cut off about 2 inches of the posterior end of the stem exposed as above, and boil it in dilute hydrochloric acid till the parenchyma is soft: for further treatment of this see below. Meanwhile smooth the cut end of the remainder of the stock with a razor, so that it may present an even surface of **transverse section,** and observe—

a. The great irregularity of outline, due to the close crowding of the bases of the leaves.

b. The dark brown band of **Sclerenchyma** bordering the periphery of the section.

c. The great bulk of the stem consisting of yellowish **Parenchyma,** with very bulky central **Pith.**

d. Round the latter a number of isolated, **large Vascular bundles,** forming an interrupted ring.

e. Outside these, and running out into the leaves,

are numerous smaller **bundles of the leaf-trace,** which appear to be less regularly arranged.

III. Divide the stock, including the apical bud, into two symmetrical halves by cutting it in a median longitudinal plane: smooth one of the cut surfaces with a razor, and observe—

a. That the stem is of almost equal thickness throughout its length, *i.e.*, it is roughly cylindrical.

b. That its external conformation is very irregular by reason of the closely crowded insertion of the leaves.

c. The bulky central Pith as before.

d. The large vascular bundles (*d* above), which are not continuous in direct longitudinal lines, but form an interrupted series.

e. The smaller bundles of the **leaf-trace** (*e* above), which in some cases may be followed, after a little careful dissection of the parenchyma which surrounds them, from one of the larger bundles of the central system into the base of one of the leaves.

Slice away carefully the external tissues of the posterior part of the stock, so as to lay bare the central system of larger bundles: it will then be seen that these form a continuous **network** with large meshes, and that each mesh is opposite the point of insertion of one of the leaves, hence it is called a **foliar gap.** Observe also that the vascular bundles of the leaf are given off from the margin of its own mesh.

IV. Confirm these observations by the dissection of the part of the stock macerated in dilute hydrochloric acid. The parenchyma, being thus rendered soft and friable, may be easily removed, leaving the vascular system as a net-work of stronger bundles, which gives

off numerous weaker bundles from the margins of its meshes: these weaker bundles run out into the leaves.

V. Remove from the apical bud the large quantities of scaly hairs (ramenta), so as to lay bare—

1. The **young leaves,** with their circinate vernation.

2. The broad **apex of the stem** with leaves in various stages of development around it.

3. The **young roots,** which will be found already present on the bases of very young leaves.

4. The **young buds,** which may be observed at a very early stage on the posterior side of the leaves.

Though the above is the general type of bundle-arrangement for Ferns with ascending or upright stems, in Ferns with creeping stems other modes of arrangement are found, which, however, may be regarded as being related to the type above described. Thus (1) in the *Hymenophyllaceæ*, &c., there is a single central bundle, an arrangement which is found also in the young seedlings of other more complex forms; (2) in species of *Davallia* and others with horizontal stems, the ring consists of two stronger bundles, one running parallel to the upper, the other to the lower surface; between these are on each side several smaller bundles, which, together with the two stronger ones, form an interrupted ring as seen in transverse section; (3) in other cases there are several (in *Pteris* two) concentric rings of bundles, which give off branches to the leaves, &c.

III.—*Microscopic investigation.*

VI. Cut transverse sections of the stock: it is hardly to be expected that a transverse section of so bulky a stem as this could be cut so uniformly thin that the structure of all the tissues could be well seen; it is better therefore to cut a number of sections, each extending over a comparatively small area, and to study the various tissues separately. Mount some in (1)

glycerine or glycerine jelly, others (2) in acid aniline sulphate, others (3) in Schulze's solution. Examine under a low power, and observe successively the following tissues, starting from the periphery of the stem:

a. An **Epidermis,** consisting of a single, somewhat irregular and ill-defined layer of cells, with dark brown outer walls: their arrangement is disturbed at the point of insertion of the **scaly hairs,** which appear as plates of cells, one layer in thickness, rising obliquely from the epidermis. Beneath this is—

b. The **Ground tissue,** which is differentiated as—

i. An outer narrow band of tissue, with rather thick, colourless, pitted walls, and cell-contents with much starch: there are no intercellular spaces.

ii. A band of **Sclerenchyma** with thick, yellow, lignified, obviously stratified, and pitted walls, cell-contents as in (i), and no intercellular spaces. This merges gradually into—

iii. The bulky central mass of ground-tissue, in which the vascular bundles are embedded. It consists of cells with comparatively thin, pitted, cellulose walls, protoplasmic contents with much starch, and with intercellular spaces.

On the external surface of those parts of the cell-walls which adjoin the intercellular spaces numerous small projecting spikes may be observed: it may be readily seen that these originate in connection with the formation of the intercellular spaces.

Internal glandular hairs are also found in the intercellular spaces; they are attached by narrow stalks to single cells of the parenchyma: the globular head contains when fresh a resinous secretion, which is soluble, but not readily, in alcohol.

c. The **Vascular bundles,** of elliptical outline;

they are embedded in the ground-tissue, and are sharply circumscribed by a narrow, light brown layer of cells without intercellular spaces: this is the **Bundle-sheath.** Among the tissues inclosed by this sheath, note that a large central mass may be distinguished as consisting for the most part of elements with large cavity, no cell-contents, and rather thick walls with a peculiar marking: this is the **Xylem.** Between this and the bundle-sheath is a broad band of tissue with thin, bright-looking walls, and with protoplasmic contents: this is the **Phloem.** Since the xylem is surrounded by the phloem, this bundle is said to be of the **concentric type.**

In the sections treated with Schulze's solution, note that the walls of the inner ground-tissue stain blue, and that starch is found in the cells: that the bundle-sheath appears browner than before, that the walls of the phloem stain blue (cellulose), and the contents yellowish: that the walls of the chief constituents of the xylem stain yellow (lignified). In the sections treated with acid aniline sulphate observe the yellow coloration of the walls in the xylem, while those of the phloem are not stained.

VII. As the vascular bundles of the leaf-stalk are better fitted for minute observation, and are better types of the concentric bundle of the Fern than those of the stem, cut thin transverse sections of the lower part of the petiole. Having previously noted with a low power that in their main features the tissues resemble those above observed in the stem, examine the structure of one vascular bundle under a high power, and starting from the periphery of it note successively—

1. The **Bundle-sheath,** a single layer of cells with yellowish walls, and yellow granular contents. N.B. There are **no intercellular spaces** in this layer, nor in any of the tissues surrounded by it.

Treat a thin section with sulphuric acid, and note that the walls of the bundle-sheath retain a sharp contour, while those o the rest of the tissues swell, and become more or less disorganised.

2. The **Phloem-sheath,** which is a band of tissue of varying thickness at different parts of the bundle, being thin at the poles of the elliptical bundle, and thicker at the sides: it consists of cells of roundish form with cellulose walls, and protoplasmic contents, with starch. Note that each of the outermost cells of the phloem-sheath is as a rule opposite one cell of the bundle-sheath: this points to a common origin of the two layers.

In the bundles of many Ferns, *e.g.*, in the Rhizome of *Pteris*, the phloem-sheath appears as a single layer of cells.

3. At the inner limit of the phloem-sheath are found elements with thick cellulose walls and narrow cavity: these constitute the **Protophloem** of Russow.

4. Internally lies the broad band of true **Phloem,** which is composed of two tissue-forms—

a. **Sieve-tubes,** which appear in the transverse section as polygonal, with their thin, cellulose walls, which are lined by a delicate protoplasmic membrane including numerous highly refractive granules.

b. **Parenchymatous cells** with thin walls and protoplasmic contents.

5. Centrally lies the **Xylem,** in which also two constituents may be recognised—

a. **Tracheides,** which appear polygonal in transverse section, and have large cavities, with no cell-contents: the walls are thick and lignified, and show a peculiar structure which will be explained by a comparison with what is seen in longitudinal sections.

b. **Parenchymatous cells,** with cellulose walls, and protoplasmic contents and starch. These cells are distributed evenly throughout the xylem, and also form a band surrounding it completely.

N.B. The parenchyma in both phloem and xylem being fundamentally of similar nature may be united under the general term **Conjunctive parenchyma.**

Cut similar transverse sections of the stem of *Pteris* or *Davallia*, and note that the outlines and arrangement of the tissues are more regular than is the case in *Aspidium*.

VIII. Cut longitudinal sections of the stem of the Male Fern. First take radial sections of the peripheral tissues, and treat as above: note—

1. The **Epidermis,** with scaly hairs.

2. The sub-jacent ground tissue, and especially the **Sclerenchyma,** consisting of cells of short, prosenchymatous form, with brown pitted walls, and cell-contents: note the gradual transition from the sclerenchyma to—

3. The colourless ground-tissue, with short parenchymatous cells, and large intercellular spaces.

IX. Then cut longitudinal sections, so as to pass tangentially through the central network of bundles: treat some sections with Schulze's solution, and mount

others in glycerine. Note the several tissues above observed in the transverse sections: they have in this section a corresponding position relatively to one another: by reason of the frequent splittings and fusions of this bundle-system the several elements will appear contorted and twisted, but this does not materially affect their general arrangement.

Examine under a high power, and observe—

A. In the **Xylem** of the bundle—

a. The **Scalariform tracheides,** which are the main constituents of the xylem: they are elongated prosenchymatous elements, with ladder-like marking of the lateral walls; this is due to the presence of regularly arranged, transversely elongated, bordered pits. Take especial notice of the appearance of the lateral walls as seen in longitudinal section where two tracheides are contiguous with one another; and compare them with parts of the wall which adjoin.

b. Cells of the **Conjunctive parenchyma** interspersed among the tracheides.

c. **Tracheides** with **spiral marking;** these are the first-formed xylem elements, or **Protoxylem.**

B. In the **Phloem** observe—

a. The **Sieve-tubes,** which are also elongated elements with pointed ends: the surfaces of the walls which separate contiguous sieve-tubes are covered with numerous **sieve-plates** (best seen in sections treated with Schulze's solution), to which round, highly refractive granules adhere: these stain yellow with Schulze's solution. Note especially the irregular outline of the walls when seen in longitudinal section.

N.B. The sieve-tubes are better seen in similar sections of the Rhizome of *Pteris.*

b. Cells of the **conjunctive parenchyma** interspersed among the sieve-tubes.

X. Separate some pieces of the vascular bundles from the surrounding tissue, and warm them gently in a test tube with potassium chlorate and nitric acid, till the elements of the bundle may be separated easily one from another; then stop the action by diluting with water, and mount in water. By preparing them in this way the tracheides, &c., may be subjected to separate examination, and their form and structure may be made out.

Apply the same process to the sclerenchyma, and observe the form and marking of the walls of its constituent elements.

XI. From around the apical bud of a well-grown plant of the Male Fern remove successively the bases of the leaves of previous years, those of the current year, and finally the larger circinate leaves, which would have unfolded in the following year. Carefully remove the smaller ones with a scalpel, and then with forceps gradually pull off the large mass of brown scales, which completely cover the extreme apex. With a camel's-hair brush remove the bases of these scales, together with the youngest of them, which will still remain round the *punctum vegetationis;* after this treatment it will be easy to observe with a pocket lens—

1. The **Apical cone** (*punctum vegetationis*), a rounded papilla, occupying a central and terminal position in the flattened apical region.

2. The **young leaves,** situated round the apical cone, and successively larger the further they are from

the apex. Note the circinate curvature which appears at an early period in their development.

XII. With a sharp razor, wet with water or with very weak spirit, remove the extreme apex of the *punctum vegetationis*, taking care to cut accurately in a transverse plane: mount first in water, and examine with a low power. If the section be thin enough, it will be seen that a cell of **triangular outline** occupies the centre of the apical cone, while the cells immediately surrounding it are arranged in more regular order than those at a greater distance. This cell is the **Apical cell,** and the cells surrounding it have been derived by cell-division from it, and are called therefore the **Segmental cells:** it may readily be seen that these again undergo subdivision.

N.B. If the section be not sufficiently transparent, it may be treated with very dilute potash and weak glycerine, which will clarify the tissues, and make the cell-walls more distinct.

XIII. From the apex of another plant cut median longitudinal sections; mount first in weak glycerine, and a little very dilute potash may be added if the sections are not transparent enough.

If any one of the sections has passed through the apical cone, in a median plane, the **Apical cell** will be seen presenting a wedge-like appearance, and the cells around it will show, in the regularity of their arrangement, that they have been derived from segments successively cut off from the apical cell. It may be concluded from the observation of transverse and median longitudinal sections that the form of the apical cell is that of a three-sided pyramid.

The structure and mode of origin of the young leaves should also be observed in the median longitudinal sections.

The Root.

XIV. Cut transverse sections of the root of the Male Fern, selecting for that purpose the thickest part of an old root: mount in glycerine, and observe—

1. There is not any well-marked epidermis: single superficial cells have grown out as root-hairs, remnants of which may still be seen.

2. The greater part of the section consists of the bulky, brown-walled **Cortex,** of which the outer parts are thin-walled; but, passing inwards, there is a sudden increase in thickness of the walls, so as to form a dense **sclerenchymatous ring:** this surrounds—

3. The **Bundle-sheath,** which consists of a single layer of cells flattened tangentially, and having the usual dotted marking of the radial walls. [N.B. This may be difficult to observe as the radial walls are often pressed out of shape.] Within this layer lies—

4. The **Phloem-sheath,** which usually consists of two layers of cells, with thin walls, and obvious protoplasmic contents. The vascular tissues inclosed within these layers are arranged according to the ordinary radial type; thus there will be seen—

5. Two groups of **Xylem** abutting on the phloem-sheath, and composed of **Tracheides** of various size, the largest being near the centre of the root: the two originally separate groups of xylem unite at the centre by formation of fresh tracheides, and together

form a flat band which traverses the root longitudinally. Alternating with the groups of primary xylem at the periphery of the vascular cylinder are—

6. Two groups of **Phloem,** consisting mainly of **Sieve-tubes** having the same characters as those of the stem. Scattered among the vascular elements are cells of **conjunctive parenchyma.**

Note that one or two cells of the bundle-sheath opposite the groups of xylem are larger than the rest: these are the **rhizogenic cells,** which might have been the starting points of lateral roots: the latter are formed at an early stage of development of the tissues of the root, *i.e.*, near to the apex; if transverse sections be made through the young part of a root, lateral roots may be found in course of development in positions corresponding to the rhizogenic cells.

If transverse sections of the root be cut at a point not far removed from the apex, it will be seen that the xylem is not yet developed at the central part of the vascular cylinder, while the peripheral parts may be fully formed: thus the development of the xylem is centripetal, the root is diarch, and the arrangement of the vascular tissues is radial.

XV. Cut **median** longitudinal sections of the apex of a root, which has been hardened in alcohol (of course at most only one absolutely median section can be obtained from a single root: it will be found convenient to embed the apex of the root in cocoa-butter, or to hold it between pieces of pith or carrot). Mount in glycerine, and examine first with a low power, and choose out those sections in which there is a symmetrical arrangement of tissues around a single, large, apparently three-cornered **Apical cell,** which lies at some distance from the extreme apex. Note—

1. That the **orientation** of the apical cell is con-

stant, *i.e.*, one corner is directed towards the older part of the root, while the side opposite that corner, *i.e.*, the anterior face or **base** of the cell, is at right-angles to the axis of the root.

2. That around the apical cell are regularly arranged **Segmental cells,** which have successively been cut off from it by walls parallel to the sides of the apical cell. Of these—

a. Those successively cut off from the base form the **Root-cap,** dividing up by regularly arranged walls into a mass of regular cells.

b. Those cut off from the sides of the apical cell form the body of the root: these also divide by walls in regular succession. Observe carefully the arrangement of these walls, and by comparison of several sections ascertain their order of succession, and their relation to the various tissues of the root above described.

XVI. Cut successive transverse sections of the apex of a root which has been hardened in alcohol. (This may easily be done if the root be held between pieces of pith, or by embedding in cocoa-butter.) If possible keep all the sections in their proper order of succession, and mount in glycerine. Examine with a low power, and choose out those in which the large apical cell is to be seen. Observe carefully—

1. The **form** of the **apical cell,** apparently **three-sided:** combining this result with that obtained by examination of the longitudinal sections; the form of the whole cell must be a **three-sided pyramid.**

2. The **Segments** are arranged in regular order round it, and are cut off successively from the three sides.

3. Note the mode in which the several segments are further divided up.

Next examine a section which has passed through the root-cap **immediately above the apical cell:** this will include the young segments cut off from the base of the apical cell by transverse walls, and destined to form the root-cap. Note the first divisions of these segments by walls arranged crosswise: it may be seen that these walls do not coincide in position in successive segments.

The Leaf.

XVII. Cut transverse sections of a pinna of a leaf of the Male Fern which has no sori upon it: mount in weak glycerine, and observe with a low power that the outline of the section shows the leaf to be of equal thickness throughout, except where traversed by vascular bundles: at those points the pinna is thickened, the lower surface projecting convexly.

Examine with a high power, and observe successively the following tissues, starting from the upper surface:—

1. A regular **Epidermis** with a thin cuticle: the epidermal cells contain chlorophyll: there are no stomata.

2. The **Mesophyll** consists in its upper part of thin-walled cells containing chlorophyll, and with small intercellular spaces; this passes by gradual transition into the lower part, where the intercellular spaces are larger, and the form of the cells less regular.

3. The lower **Epidermis,** the cells of which also contain chlorophyll: numerous **Stomata** are present:

note the form of the two **Guard-cells** as seen in transverse section, and their position in relation to the epidermis.

4. Here and there **Vascular bundles,** of circular appearance in transverse section, will be found embedded in the mesophyll: the larger of these correspond in position to the swollen ribs of the pinna.

Note the **Bundle-sheath** as a continuous layer of cells, which completely surrounds the circular bundle, and within this the xylem and phloem elements similar to those of the stem: the bundles show a tendency to the **collateral** type, the xylem being nearest to the upper surface of the leaf.

XVIII. Cut tangential sections (or strip off the epidermis) from (*a*) the upper, and (*b*) the lower surface of the leaf: mount as before, and compare them.

(*a.*) The epidermis of the **upper surface** will be found to consist of cells with sinuous outline, and protoplasmic contents, with chlorophyll: **no stomata** will be found.

(*b.*) The epidermis of the **lower surface** consists of cells similar to the above; there are **stomata** with two guard-cells.

The development of these stomata may be studied with advantage in young leaves, by stripping off the epidermis, or by cutting tangential sections. Special attention may be given to the peculiar case of *Aneimia.*

The Sporangia.

XIX. Cut transverse sections through pinnæ of leaves which bear **Sori,** taking care that the sections shall

pass through one or more sori. Mount as before, and examine with a low power. Note—

1. The structure of the pinna, as above described.

2. Opposite to, and seated upon a rib will be found the membranous **Indusium,** which, like an umbrella, covers over—

3. The **Sporangia,** which are biconvex-lens-shaped, brown, stalked capsules, attached to the rib, and filled with—

4. Numerous roundish, brown, unicellular **Spores.**

Observe more closely the structure of the single sporangium. It is composed of—

i. The **Stalk,** which is of considerable length, and usually consists of three rows of cells.

Stalked glandular bodies are often found as lateral branches on the stalk of the sporangium in this species.

ii. The **Capsule,** which has the form of a biconvex lens, and consists of a marginal series of cells with thickened walls, which constitute the **ring,** and thinner-walled, flattened cells, which together form the lateral walls of the completely closed sporangium.

Note sporangia in which the lateral walls have been ruptured transversely, the ring having straightened itself out with sufficient force to tear the more delicate lateral walls: by this means the spores are liberated.

Examine single spores under a high power; they are **unicellular** bodies, having a brown wall, with external band-like outgrowths of the exospore or outer layer of the wall. All the spores are alike (Homosporous).

The various stages of development of the sporangium may be found in any sorus in which only the first sporangia have come

to maturity. Note especially in such sori the following stages of development :—

i. A simple, hair-like process, consisting of a single cell, or of two separated by a transverse wall.

ii. The upper cell divided up so as to consist of a central tetrahedral cell (**archespore**), surrounded by a single layer of cells, which form the wall.

iii. The central cell or archespore is divided into—

a. One cell, or a group of cells, lying centrally, which gives rise to the mother-cells of the spores, and finally to the spores themselves.

b. A layer of transitory **tapetal cells,** which surround *a*, and are ultimately absorbed.

B.—THE OOPHORE.

I. Dry some of the leaves of the Male Fern, which bear sori, on a piece of paper: the spores will then be set free by the rupture of the sporangia, and they may thus be collected in large quantities. Sow some of them on damp earth: keep them moist, and sheltered from direct sunlight: they will then germinate, and after a few weeks the surface of the soil will be found to be covered with small, green, flattened bodies, each of which is an individual **Prothallus.**

If it be desired to follow the germination of the spore, and the first stages of the development of the prothallus in detail, the spores may be placed in a hanging drop in a moist chamber, as described, p. 16. But for all ordinary purposes it will suffice to pick off young prothalli, from time to time, with a needle from the surface of the soil on which spores have previously been sown: by this means a series of preparations illustrating the various stages of development of the prothallus may be obtained. Note in such a series of preparations, under a low power—

1. The bursting of the outer coat of the spore, and the protrusion of the inner coat through the slit.

2. The formation of—

a. An aerial portion, containing chlorophyll, and undergoing repeated cell-divisions, which result in the development of a flattened, roughly triangular, expansion.

b. A root-hair, which remains undivided, does not contain chlorophyll, and grows downwards into the soil.

3. In older prothalli of the series note an incurving of the margin of the part more remote from the original spore, this is due to the slower growth of that part and the more rapid growth of the lateral parts; at the base of this depression is one wedge-shaped **apical cell,** from which segments are cut off alternately on opposite sides.

N.B. The identity of the apical cell and regularity of the segments are lost in the later stages of development.

II. Examine a single prothallus with the naked eye, and observe—

1. The **form,** which is flattened, and more or less kidney-shaped, with a depression of the margin, at the base of which is the **Organic apex** of the prothallus. Note that the central part of the prothallus is often perceptibly thicker than the periphery: this thicker part is called the **Cushion.**

2. The **position** of the prothallus while growing; it is usually oblique to the surface of the soil.

3. The **Root-Hairs,** which spring from the under surface of the cushion, and run downwards into the soil.

4. The **green colour,** due to the presence of chlorophyll: the prothallus is thus capable, under suitable circumstances, of carrying on the process of elaboration of fresh organic substances.

III. Wash a fresh, well-developed prothallus carefully in water, so as to remove the soil from the root-hairs:

mount it whole, in water, with the lower surface directed upwards, and examine it with a low power. Observe again the chief points above noted with the naked eye which are now more plainly seen, and note especially—

1. The **form** and **structure** of the cells in the lateral, thinner portions of the prothallus; they are polygonal, and have thin cellulose walls, and protoplasm containing a nucleus, and numerous chlorophyll grains: the cells at the margin are often extended as hair-like outgrowths.

2. The cells composing the cushion are of similar structure, but are aggregated in a mass more than one layer of cells in thickness: many of the cells will be seen to have grown out as root hairs.

3. The depressed **Apex** of the prothallus, which is occupied, not by a single wedge-shaped cell, as is the case in early stages of development [see above, small type, p. 205], but by a closely aggregated series of marginal cells, with thin cell-walls, and every appearance of recent and repeated cell-divisions.

4. The **Antheridia,** which are hemispherical outgrowths, situated chiefly on the posterior and lateral portions of the under side of the prothallus.

5. The **Archegonia,** which are situated on the cushion near to the organic apex of the prothallus; the multicellular **Neck** of the archegonium projects from the surface of the prothallus as an elongated cylindrical structure.

Under the low power select one mature antheridium, and, without moving the slide, adjust the higher power so as to observe the structure of the same antheridium in detail. It will then be seen that it consists of—

a. A **wall,** composed of a single layer of narrow cells; this completely surrounds—

b. The **mother-cells of the Antherozoids,** which are small, and not very numerous.

Other antheridia may be found which have already burst the outer wall; in these the contents of the mother-cells may perhaps be seen escaping from the ruptured antheridium as spiral **Antherozoids,** endowed with active movements.

If a preparation showing motile antherozoids be treated with a weak solution of iodine, the movements will cease with the death of the antherozoids, which will assume a brown staining, while the **cilia** attached to the anterior ends of them will then be clearly seen.

Select under the low power one mature **Archegonium,** and then observe it in detail under the higher power. If the neck be vertical, which would under the circumstances be the natural position, there will then be seen, on focussing down upon it, **four** cells composing the wall of the neck, and surrounding one cell, the **Canal cell.**

IV. Treat some prothalli with a saturated solution of picric acid in water for some hours. Wash them with water, and then harden them gradually by successive treatment with alcohol of 50 per cent., 70 per cent., and finally with absolute alcohol or strong methylated spirit. [N.B. The preparations described below may also be made from fresh material, but the results will not be nearly so good as if the above method of fixing and hardening be adopted.]

Hold a prothallus thus prepared between pieces of pith, or imbed as directed on p. 4 &c.; then cut

sections perpendicularly to the surface of the prothallus, and so as to pass through the cushion, following the organic axis from base to apex. Mount in one part glycerine and one part water, and examine first with a low power.

The **lower surface** may easily be recognised by the presence of **root-hairs,** and on this lower side, chiefly near to the apical end of the section which is characterised by its small cells with thin walls, will be found **Archegonia**; these may be recognised by the multicellular **neck,** which projects beyond the surface of the section. [N.B. In some cases the canal of the neck may appear of a deep brown colour: these are old archegonia which have not been fertilised, and they must be disregarded.] Select one **archegonium** of full size and healthy appearance, and examine it under a high power.

Observe that it consists of—

A. A **Central series** of three cells, which may be distinguished as—

a. The **Canal-cell:** this is oblong in form, and its walls are subject to mucilaginous degeneration; it occupies the channel of the neck, and has been above alluded to as being visible when the neck of the archegonium is seen from above.

b. The small **Ventral Canal-cell,** which lies immediately below the oblong canal-cell, and is of rounded form.

c. The **Oosphere,** which is of relatively large size, and roughly spherical form; it is embedded in the tissue of the cushion, and consists of a dense mass of granular protoplasm.

B. A **Neck,** which is composed of cells arranged in four rows, constituting together a cylinder or tube, one layer of cells in thickness: this projects from the surface of the prothallus, and incloses the cells (*a*) and (*b*) of the central series, while (*c*) the oosphere is embedded in, and surrounded by, cells of the cushion.

At the end of the section more remote from the apex may be found **Antheridia.** Select one fully developed, and it will be seen to consist essentially of an outer wall, one layer of cells in thickness, which incloses a central mass of cells, the contents of which may be seen to be rounded off, and to have assumed the form of a closely-coiled spiral: these are the **mother-cells of the antherozoids.**

By comparing carefully-prepared and well-cut sections, the development of the antheridia and archegonia may be traced, and in both cases it may be seen that they originate from single superficial cells. In the case of the antheridia young stages of development are to be found on sections through the lateral and posterior parts of the prothallus, while young stages of development of the archegonia lie near to the organic apex. Young archegonia should also be observed from above in young prothalli mounted with the lower surface uppermost. If drawings be made of archegonia from both points of view, and of various ages, a comparison of them will give a clear idea of the processes of development.

The dehiscence of the antheridia, the escape of the antherozoids, and their movements, should be observed with particular attention in fresh prothalli mounted in water; also the opening of the apex of the neck of the archegonia: in both cases the process depends upon a mucilaginous degeneration of cell-walls of the inner cells, and a subsequent swelling by taking up water, and consequent rupture of the outer walls. Further, the movements of the living antherozoids may be followed, and the act of fertilisation observed; the antherozoids being arrested by the

mucilaginous mass projecting from the open archegonium, they pass through this and down the neck of the archegonium, and finally coalesce with the oosphere.

C.—*THE YOUNG SPOROPHORE, OR FERN PLANT.*

V. The result of the process of fertilisation of the oosphore of the archegonium by the antherozoids is the development of a new Fern plant, or Sporophore, and in cultures which have been continued for some months such young Fern plants may be clearly seen attached to the prothalli, but one prothallus produces only one young Fern plant.

Select a prothallus to which a young Fern plant is thus attached, and wash from it the soil which adheres to it. Examine it with a lens, and observe—

1. That the prothallus itself is similar in form and structure to those before observed.

2. That the young Fern plant is firmly attached to its under surface by a lateral protrusion (**Foot**).

3. That the young Fern plant consists of the following parts:—

a. A **Root** which turns downwards into the soil.

b. A lateral protrusion, the **Foot,** which maintains a close physiological connection between the prothallus and the Fern plant.

c. A first leaf, or **Cotyledon,** with an elongated petiole, and bifurcating, expanded, upper part: this usually grows upwards through the depression at the apex of the prothallus.

d. Between the base of the cotyledon and the foot is the **Apex of the stem,** which continues its growth, and produces new leaves.

Having thus gained a knowledge of the position of the several parts relatively to one another, and to the prothallus, in the case of a young Fern plant of considerable size, younger plants may successively be taken, and by a comparison of these the mode of **development** of the young embryo, or Fern plant, may be traced. In order to make preparations so as to show the several parts of the embryo, sections should be made either from fresh material, or better from material prepared with picric acid, and hardened in alcohol, as above directed for the prothalli. The direction of section should be parallel to the organic axis of the prothallus, and perpendicular to the flattened surfaces: in such sections, including embryos of suitable age, the stem, cotyledon, root, and foot may all be observed; further, the origin of these several parts from definite segments of the fertilised oosphere may be traced. For details as to the sequence of cell-divisions in the first stages of development of the embryo reference should be made to Text-books.

C.—EQUISETINEÆ.

EQUISETUM ARVENSE (The Common Horse-Tail).

THE SPOROPHORE.

The Vegetative Organs.

I. Observe with the naked eye the following external characters in specimens of *E. arvense* which have been carefully dug up. N.B. The root-stock being a creeping one, and underground, it cannot be removed from the soil without injury by merely pulling it up: the specimens should be carefully dug up, so that the several parts may be seen in their natural position relatively to one another.

1. The external conformation of the **Axial structures** or stems is the same whether they be creeping and underground, or erect and aerial: they consist of more or less elongated joints or **Internodes,** marked off from one another by **Nodes,** which may be recognised as the points of insertion of—

2. The **Leaf-sheaths,** each of which surrounds the base of the internode next above it, and splits at its upper limit into **Teeth,** the number of which varies on different axes.

3. The internodes are marked by projecting longitudinal **Ridges,** which may be traced upwards into the

leaf-sheath, and are then seen to be continuous to the apices of the teeth: between the ridges are depressed channels.

4. The **Lateral branches** are always inserted at the nodes, and at the base of the leaf-sheaths; note that they are arranged in whorls, and appear to burst through and rupture the leaf-sheath near to its point of insertion on the axis, and at points alternating with the projecting ridges, *i.e.*, at the channels.

5. The **Roots** (to be clearly distinguished from the underground root-stock, which shows an alternation of nodes and internodes as above described), are thin and fibrous, and branch **monopodially**: they are inserted with a whorled arrangement at the nodes, immediately below the point of insertion of the lateral buds. The underground stems and the roots are covered externally by numerous fine root-hairs of a brown colour.

6. Note that at many of the nodes the lateral branches, or the roots, or both may be partially suppressed, their development being arrested at an early stage: also that frequently the basal internode of lateral shoots attached to the nodes of the root-stock may be much distended, while its apical bud is arrested: in some cases more than one internode may take part in this development, the result being a moniliform structure: the **Tubers** thus formed are reservoirs of reserve material, and being easily separated from the parent plant, they serve to propagate the plant by a purely **vegetative** process. Any node separated from the parent plant may also serve the same purpose under favourable circumstances.

7. Observe particularly that the teeth of each leaf-

sheath correspond in position to the channels of the next higher internode; since the teeth are continuous downwards with the ridges of the lower internode, it follows that the ridges of the lower internode alternate in position with those of the internode next above it.

Strip off carefully one leaf-sheath, and it may then be clearly seen that the ridges of the upper internode alternate with those of the internode next below it.

II. Cut transverse sections from a mature internode of an upright aerial stem: mount some in glycerine, others in Schulze's solution, and examine first with a low power: observe—

i. The sinuous outline of the section, the projections corresponding to the ridges observed externally with the naked eye, and the indentations to the channels intervening between them.

ii. The section is limited at the periphery by an ill-defined layer of epidermis, which, together with subjacent tissues, forms a **band of thick-walled tissue** of very variable breadth; thus the band is broad at the most convex parts of the ridges, and at the most depressed parts of the channels, while it is reduced on the sloping sides of the ridges to the single layer of thick-walled epidermal cells.

iii. Beneath this is a broad band of **Cortical tissue,** in which may be recognised—

a. Groups of **Chlorophyll-parenchyma** of oval outline; one of these lies opposite to each of the ridges, and extends to points close below the surface of the sloping sides.

b. Parenchyma composed of rounded cells with little or no chlorophyll.

c. Large **Intercellular cavities,** which alternate in position with the ridges, and are thus opposite the channels of the outer surface.

iv. The cortex is limited internally by a single sinuous layer consisting of cells in close contact with one another; this is the **Bundle-sheath** (see below): it forms a continuous and sinuous ring surrounding—

v. The **Vascular bundles,** which may be recognised as oval groups of elements of smaller size than those of the surrounding tissue: they alternate in position with the intercellular cavities of the cortex, and are thus **opposite to the ridges** which project on the external surface.

vi. The **Pith,** which lies centrally, consists of thin-walled tissue, and is in great part obliterated by a large central cavity.

III. Before proceeding to the more minute study of these several tissues, cut transverse sections through a leaf-sheath: mount in glycerine, and examine with a low power. It may be observed that the arrangement of tissues is not unlike that of the peripheral tissues of the internode. Note especially that as in the internode, so also in the leaf-sheath, one vascular bundle (here of small size and simple structure), is to be found opposite each ridge.

IV. Cut a series of rather thick transverse sections through the nodal region: it will be best to select one which bears no fully-developed, lateral branches. **Keep them all in their right order of succession,** mount, and compare them under a low power, starting from such a section above the node, as will show an arrangement of tissues typical of the internode, together

with the leaf-sheath surrounding it. If these parts be in their natural position, it will be seen in this first section that the vascular bundles of the leaf-sheath alternate in position with those of the internodes. Passing the sections successively under observation, it will be seen that each bundle of the internode divides into two branches, which diverge and insert themselves respectively right and left on bundles of the leaf-sheath, at the point where these curve into the axis, and begin their downward course through the next internode. Thus the course of each bundle of the leaf-trace is simply this: it passes from the leaf-sheath into the axis, traverses one internode, and at the next lower node it forks, the branch bundles inserting themselves on bundles entering at that node.

These facts, together with external observation of the ridges of the leaf-sheaths and internodes, will suffice for the construction of the whole bundle-system of the shoot of *E. arvense,* which is also typical of the whole group.

The bundle-system may be actually demonstrated by dissection in the stem of one of the larger forms, viz., *E. Telmateia.* Take a fresh and well-grown shoot, and cut from the thickest part of it a piece about four inches in length, and including a node: then remove from it the outer tissues, and scrape the soft parenchyma away till the vascular bundles are laid bare; then slit the hollow stem longitudinally, flatten it out, and carefully scrape away the softer tissues from the inside till the vascular bundles are clearly seen; then treat for some hours with alcohol to remove the air bubbles from the intercellular spaces, and warm gently in weak solution of potash: the preparation may be preserved in glycerine, or in glycerine jelly. If such a preparation be carefully made it will show the course of the vascular bundles in the internode, as well as the branchings and fusions at the node.

Returning to the study of the transverse sections of the internode, examine them under a high power, and observe—

1. The superficial cells of irregular size and shape which form an ill-defined epidermis, many projecting as rounded excrescences beyond the general surface: their walls are thick, and show on the outer surface small and irregular projections: the cell-contents are scanty. Note that on the sloping sides of the ridges, and immediately above the chlorophyll-parenchyma, **Stomata** may be seen cut in section, and showing two **Guard-cells** which surround the pore, and two **Subsidiary cells** which fit closely round them: there is a large respiratory cavity beneath each stoma.

2. The sub-adjacent cells, composing with the epidermis the band of thick-walled tissue before mentioned, have cellulose walls (blue with Schulze's solution), with narrow pits.

3. The cells of the **Chlorophyll-parenchyma** are thin-walled, and of oblong form: the chlorophyll granules are numerous and clearly marked.

4. The remnants of disorganised cells along the margins of the intercellular cavities, which show that they are of **lysigenetic** origin: the same may be observed with regard to the central cavity.

Add a little caustic potash to the sections mounted in glycerine, and then observe the cells of the bundle-sheath under a high power: their radial walls will be seen to show the characteristic dark dot-like appearance. Passing on to the **Vascular bundles,** their most marked constituents will be two to four groups of dark-looking elements, which are **Tracheides**

of the Xylem, and are disposed, roughly speaking, in the form of a V, while the apex of the V is occupied in each bundle by a large **air-cavity.** There are originally four groups of xylem elements in each bundle, two bordering on the cavity, and two nearer the bundle-sheath; the elements of the former are often only imperfectly seen in transverse sections, since they are apt to become disorganised during development. Between the air-cavity and the bundle-sheath lies a mass of tissue of the **Phloem,** with relatively thin cellulose walls; **Sieve-plates** may sometimes be observed in surface view in this tissue.

V. Compare transverse sections of the underground axis, or root-stock, with those of the aerial axis: the sections may be prepared in the same way as the above. Note that—

1. The superficial cells have brown walls, and often grow out as long, brown, root-hairs: there are no stomata.

2. The sub-jacent cortex is thin-walled, and colourless, and often contains much starch.

3. The ridges, intercellular cavities of the cortex, and vascular bundles have the same relative positions as in the aerial stem.

4. The structure of the vascular bundles is similar to that in the aerial axis.

5. There is no cavity at the centre of the axis.

Cut transverse sections of one of the tubers; treat with potash, and mount in glycerine. Observe—

1. The brown-walled **epidermis** with many hairs, similar to those on the root-stock.

2. A **sub-epidermal layer** with thickened walls.

3. The bulky **parenchyma** with numerous starch grains: there are no large intercellular cavities as in the aerial axis.

4. Isolated **vascular bundles,** each of which is surrounded by a **special bundle-sheath,** quite distinct from that of the other bundles; there is no general bundle-sheath as in the normal axes.

A comparison of the stems of various species of *Equisetum* in respect of the bundle-sheath shows that there is some want of uniformity in its arrangement, even in the normal axes: further, it has been shown that such differences may occur between the rhizome and the aerial axis even in the same species. In *E. arvense* there is a difference in this respect between the tubers and other axes.

VI. Make preparations suitable for the study of the epidermis in surface view, by cutting longitudinal, tangential sections, and treat as before, mounting with the outer surface uppermost: observe under a high power—

1. That the superficial cells covering the ridges are of elongated form, with smooth outer walls, and thickened, pitted, inner walls: there are no stomata on the ridges.

2. That the superficial cells of the grooves are shorter, and nearly square, their outer walls bearing those rounded excrescences already observed in transverse sections, while their whole surface is dotted with small projections: in this part are also numerous **Stomata,** which present the characteristic appearance of two concentric circles, the outer being the limit of the **two subsidiary cells,** the inner that of the **two guard-cells.** Note also the peculiar radiate marking, which is due to irregularity of thickening of the wall separating the guard-cells from the subsidiary cells.

Treat sections similar to the above with Schulze's macerating fluid ($K\,Cl\,O_3 + H\,N\,O_3$) for some hours, and then dry them with

blotting-paper, and ignite them in a spirit lamp on platinum foil, or on a cover glass; then treat the ash with weak acetic acid; mount the residue, and examine under a high power: a **skeleton** will then be found to remain, which represents clearly the several details of structure of the epidermis above described. From the treatment which the preparation has undergone it may be concluded that this is a **skeleton of silica.**

VII. Cut radial longitudinal sections of an internode of an underground stem: wash them well with water to remove as much as possible of the starch, and mount some of them in glycerine, others in Schulze's solution. Note successively the following tissues—

1. The oblong superficial cells with brown walls, frequently bearing unicellular hairs.

2. The oblong cells of the **Cortex** with cellulose walls, and containing starch.

3. The **Vascular bundles,** which may be easily recognised as transparent bands of tissue, in which may be clearly seen—

a. The elongated **Tracheides** of the xylem, showing **annular, spiral,** or irregularly **reticulate** thickening of the walls: these thickenings stain yellow with Schulze's solution: there are no protoplasmic contents: the lignified rings are often found free in the intercellular cavities, owing to the rupture of the thinner parts of the walls: for this reason also the annular vessels, which adjoin the intercellular cavities in the bundles, are frequently not to be found in transverse sections.

b. The **Phloem** consisting of—

a. **Sieve-tubes,** which are elongated elements, with cellulose walls, and granular protoplasmic contents,

and are divided into joints by transverse or oblique walls: they correspond in general characters to the sieve-tubes of the higher plants, but the sieve-structure of the terminal walls is not clear. Numerous highly refractive granules are found on both sides of the terminal walls.

β. **Cambiform cells** of oblong form, with cellulose walls.

VIII. From buds which have been hardened in alcohol cut median longitudinal sections: treat them for a short time with a strong solution of caustic potash, then wash them with water, and mount in strong acetic acid.

Examine them first with a low power, and observe that the **nodes** and **internodes** are easily recognised in the lower, older parts of the sections; the former being the points of insertion of the leaf-sheaths, opposite which are various complications of the arrangement of the tissues as has already been observed; in the internodes the tissues show greater regularity of arrangement. Note that on passing towards the apex the internodes are successively shorter, and the character of the tissues of both nodes and internodes becomes more uniform; also that the leaf-sheaths become successively shorter. Following the axis upwards it may be seen to terminate in a sharp cone, which is the *punctum vegetationis*, consisting of cells undergoing division, which constitute the **primary meristem.** Here and there it may be seen that **lateral buds** have been cut through, they are situated at the nodes, and appear to be completely surrounded by the tissues at the bases of the leaves; in their form and structure they resemble the *punctum vegetationis* of the main

axis, but on a smaller scale. Note also the irregularly annular or spiral tracheides in the internodes, and the way in which their structure is modified at the nodes, where they appear shorter, and are more closely reticulated.

Examine the *punctum vegetationis* under a high power, and observe—

1. At the extreme apex a single, large, wedge-shaped cell; this is the **Apical cell.** The cells immediately adjoining it are arranged in regular order, and are of definite form, being **Segments** successively cut off from the apical cell. Observe how the older segments, which are further from the apical cell, have been successively divided up by walls perpendicular to the outer surface (**anticlinal**), and parallel to the outer surface (**periclinal**). The details of arrangement of the successive walls may with advantage be traced by comparison of several preparations, and explained by reference to the Text-Books. Since the superficial cells are subject to repeated periclinal divisions it is clear that there is no definite layer of dermatogen: compare this structure of the *punctum vegetationis* with that of the lateral buds above mentioned.

2. Note the leaf-sheaths, successively smaller towards the extreme apex, and observe how they originate by outgrowth and division of successive zones of cells below the apex.

3. Attention should also be paid to the mode of **origin of the lateral buds:** a diligent comparison of them in various stages of development will show that they are not of endogenous origin, but are derived from superficial cells lying immediately above the insertions

of the leaf-sheaths. These cells divide, and form the young buds, which subsequently appear to be completely embedded in the tissue of the leaf-sheath, and ultimately burst through it.

4. It will be useful further to trace the development of the several tissues, and to note their relations to the apical cell and its segments.

IX. Cut a series of transverse sections through a bud: prepare and mount them as above directed (VIII), being careful to keep them in their proper order of succession, and with their upper side uppermost.

Some of the sections will only have passed through the upper parts of the leaf-sheaths, which will appear as concentric rings, with a structure similar to that already observed (III.): note that the leaves of successive whorls alternate one with another. In the centre of these rings there will be found in each of the lower sections of the series a transverse section of the axis, and one of the sections should include the *punctum vegetationis*, which would thus be **seen from above.** In this preparation observe that the **apical cell** appears of **triangular outline,** while the segments are arranged regularly around it: from this observation, and from its appearance in the longitudinal section, it may be concluded that the apical cell has the form of a **three-sided pyramid,** and that segments are cut off from three sides. From the observation of transverse sections cutting the axis below the apical cell, and a comparison of these results with those drawn from a study of longitudinal sections, the mode of subdivision of the segments should be fully made out.

X. Cut transverse sections of a well-developed root

of *E. arvense*, treat them with potash, and mount in glycerine: examine them under a high power, and observe—

1. That there is a peripheral band of tissue with dark brown walls: single superficial cells have grown out as root-hairs.

2. Then follows a broad band of colourless **Cortex,** with large intercellular spaces; this is limited internally by—

3. A definite layer of cells having the well-marked characteristics of the **Bundle-sheath:**

4. Within this is the **Phloem-sheath,** the cells of which are opposite to those of the bundle-sheath, and are derived with the latter from the inmost layer of the cortex. This surrounds—

5. The vascular cylinder, consisting of—

a. Four **Xylem** groups, each of which may consist of only one tracheide, while one large element often occupies a central position.

b. The spaces between these are occupied by ill-defined groups of **Phloem,** and **Conjunctive parenchyma.**

The arrangement of tissues at the apex of the root of *Equisetum* may be studied in the same way as above described for the root of *Aspidium Filix-Mas*, and it will be found to be similar to it in all the more important points. Attention should also be paid to the **mode of origin of the lateral roots,** which here spring from the phloem-sheath, while in Ferns they arise from cells of the bundle-sheath.

The Sporangia.

XI. Examine one of the fertile stems, which rise above ground in the spring, with the naked eye; ob-

serve that the internodes and leaf-sheaths of the lower part of it are similar to those of the vegetative axes. Passing upwards, note that the last leaf-sheath below the spike is of smaller size than the rest. The spike itself is covered by closely-arranged peltate scales, of hexagonal outline as seen from without: these are arranged in more or less regular whorls.

Remove some of the scales, and examine one of them in detail: it consists of a thin pedicel by which it is attached to the axis; the pedicel widens out towards its apex into a flattened shield-like structure, from the lower surface of which a number of sacs (**Sporangia**) are suspended.

XII. Cut transverse sections through a spike, so as to include some of the scales: mount in glycerine, and observe under a low power. There will be seen a bulky **Pith,** a ring of **Vascular bundles,** and a band of cortex. The **Pedicels** will appear extending radially from the axis, and widening at the outer limit into the peltate expansion, on the lower surface of which two sac-like **Sporangia** may be seen.

Note that a vascular bundle runs up the pedicel, and ramifies in the peltate expansion.

Examine one of the sporangia under a high power, and note—

a. The **Wall** which is one layer of cells in thickness: the walls of these cells are strengthened by a spiral or annular thickening: the wall ruptures by a longitudinal slit on the side next the pedicel.

b. Many **Spores** may be found in the sporangia, or scattered through the glycerine: examine them carefully, and observe the spirally-coiled **Elaters,** and the

smooth inner coats of the spore, which inclose a protoplasmic body with a well-marked nucleus.

Scatter fresh spores upon a slide, and breathe upon them gently; then observe them under the microscope: the elaters will be seen to execute active movements, thus showing that they are hygroscopic.

By cutting transverse sections of spikes of various stages of development, which have been hardened in alcohol, or in picric acid and then in alcohol, mounting them in glycerine, and comparing them, the history of the development of the sporangium may be traced. The chief points to be observed will be (1) that the sporangia appear as **multicellular** protuberances. (2) A single hypodermal cell, the **archesporium,** gives rise by division to the spore-mother-cells, while the superficial layer of cells which covers the archesporium divides into three, of which the outermost alone remains as the wall of the mature sporangium. (3) Each of the spore-mother-cells divides into **four** cells, which develop further into mature spores.

THE OOPHORE.

The fresh spores may be sown on moist soil, and the first stages of germination, which are rapid, may be easily observed; the later stages are, however, slow, and to see these the cultures must be carefully kept. The result is the formation of prothalli (**oophores**) of irregular form, some of which produce **antheridia** after five to six weeks. Other prothalli of larger size produce **archegonia** after about two to three months. The antheridia are embedded in the tissue of the prothallus, and produce large antherozoids. The archegonia are borne on the upper surface. The result of fertilisation of the egg-cell of the archegonium is the formation of an embryo, which develops as the spore-bearing plant or **sporophore.**

Endeavours should be made to obtain healthy cultures of the prothalli of **Equisetum** in which the above and other points described in other Text-books may be observed.

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